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LIVER

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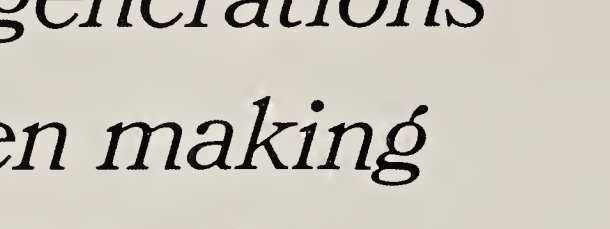

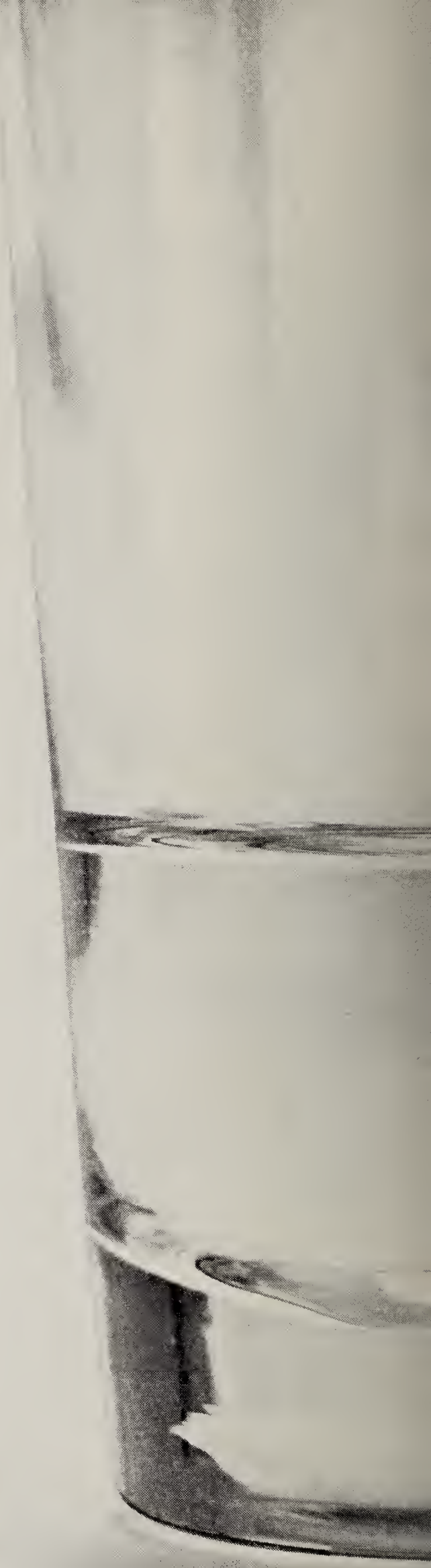

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Guest Editorial

Dr. Popper was born on November 24, 1903 in Vienna, Austria. He attended the U. of Vienna where he obtained degrees in Medicine and Pathology, M.D. 1928, and the University of Illinois, M.S. 1941, Ph.D. 1944.

From 1942-57 he served as Director of Laboratories and Pathology for Cook County Hospital and Scientific Director of the Hektoen Institute for Medical Research in Chicago.

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An Associate Editor of Gastroenterology, *1962-66, he is currently Editor of* Virchows Archiv A *and member of the Editorial Board of* Cancer Research, Experimental and Molecular Pathology, *and* Human Pathology.

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HANS POPPER, M.D., Ph.D.
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Do We have The Answer To The Riddle Of "Hepatitis?"

It is now the fashion to challenge the relevance of basic science investigations into areas far removed from immediate and social applicability. Discoveries, however, may solve relevant problems even though the original target of the study has no relation to the problem. The discovery of Australia antigen by Blumberg promises an etiologic test for the most frequent type of hepatitis, at least in adults, namely viral hepatitis. This disease is particularly frequent in the disadvantaged population and in "drug (ab)users." Australia antigen is a blood factor combining with antibodies in the sera of persons who have received large blood transfusions and thus have presumably acquired antibodies against various antigens in the donor's blood. It has been found in relatively high incidence in the populations of some Eastern countries. In the Western world, it occurs in high incidence in leukemia, in Down's syndrome and in viral hepatitis. It seems to be more frequent in acute serum than infectious hepatitis and also in some forms of chronic hepatitis. It is still too early to say to what degree it depends on genetic factors, the study of which was the stimulus for its discovery, and whether it will provide a practical test for viral hepatitis, or at least serum hepatitis. It will not be too soon because the uncertainty whether in a given patient with acute or chronic liver disease, viral hepatitis causes the disorder has clouded not only diagnosis but also management and prognosis, when a mechanical biliary obstruction, known parasites or bacteria, or rarely, a metabolic disorder has been excluded. This uncertainty entails also the etiology of cirrhosis, so frequent in the tropical countries, but recently the fourth or fifth most common cause of death in persons over 40 years of age in the U.S. This doubt has recently interfered with the evaluation of drug induced liver injuries, particularly those of the "hepatitic" type, which have a high mortality rate in the few patients in whom it develops. It creates doubt as to the safety of many useful drugs, e.g. the excellent anesthetic halothane. Finally, it makes blood transfusion hazardous. Since in the minds of many physicians "hepatitis" implies viral hepatitis until proven otherwise, it might be useful to briefly reflect on it from a microbiologic, pathologic and clinical viewpoint.

The microbiologist knows well a variety of hepatitides. It includes some forms of viral origin, such as yellow fever, Q-fever and viral hepatitis in animals, in all of which the etiologic agent is identified. However, he has been less lucky with human viral hepatitis. From epidemiologic studies, he is sure of the entity, particularly since transmission to man with infectious material has succeeded, but not to animals, with the possible exception of a South American primate, the marmoset. The etiologic diagnosis in the indi-

vidual patient depends on epidemiologic evidence which is strong in epidemics, suggestive after blood transfusions, nontherapeutic parenteral use of drugs, or intake of potentially infected food, like raw clams, and a suspicion in the sporadic case.

The pathologist has even greater problems, particularly if he desires a functional interpretation. Again, we do not consider here focal suppurative or granulomatous hepatitis but the diffuse disorders in which alterations and frequently necrosis of the hepatocytes are associated with an intralobular and portal tract inflammation. In acute hepatitis, the functional manifestations mirror the hepatocellular damage, not the inflammation. The characteristic symptoms, fatigability and malaise, reflect liver cell dysfunction rather than inflammation. The same holds true for the abnormal results of hepatic tests like elevated activities of the transaminases. The light microscopist thus cannot well correlate the histologic picture with the functional deficit because the loss of hepatocytes accounts for the hepatic insufficiency only in massive necrosis. By contrast, focal necrosis, despite its impressive appearance, on account of the associated inflammation, does not explain the functional deficit. Electron microscopy made us understand that in the absence of massive necrosis, alterations of the organelles of the viable hepatocytes, subtle under the light microscope, cause the functional deficit. Since thus in acute hepatitis the functional emphasis is on the hepatocytic damage, the German school uses such terms as hepatosis or hepatic necrosis. For good reasons this has not spread into the Anglo-American nomenclature. The first is the better communication with the clinician who is used to calling acute diffuse liver disease "hepatitis". Moreover, hepatocellular injury, if severe enough, causes a hepatic inflammation, and this permits us to separate milder hepatocellular defects from significant ones, e.g. alcoholic steatosis from alcoholic hepatitis with its strong tendencies to cirrhosis formation. Chronic hepatitis is even more confusing since the epidemiologic history is even less reliable and since it is preceded only rarely by a documented episode of acute icteric hepatitis. The inflammatory component here is functionally significant being one of the keys to the dreaded progression to cirrhosis. It accounts for the fibroplasia resulting in ominous septa formation and also for abnormal immunologic reactions which have diagnostic significance, but so far no established pathogenetic role. Self-perpetuation of the inflammatory reaction is considered the underlying process and is the target of the main therapy, namely steroids and immunosuppressive agents. The demonstration of the Australia antigen in some of these patients may alter pathogenetic, diagnostic and therapeutic principles.

Finally, let us turn to the clinician who designates as acute hepatitis disorders with varying degrees of hepatic functional deficiency and jaundice, from the anicteric to the cholestatic form. He assumes it to be viral hepatitis if he can exclude alcoholic hepatitis and drug induced hepatic injury. He can identify by liver biopsy alcoholic hepatitis, icteric or nonicteric. He has less luck with biopsy in drug-induced hepatitis and depends mainly on the history; he is sometimes helped by immunologic reactions such as antimitochondrial antibodies. He is justified to call it hepatitis, but is in need of confirmation.

The study of liver diseases, some wish to dignify it with the term hepatology, has been hampered by the uncertainty over viral hepatitis. These studies, fascinating by their progress in biochemical, biophysical and morphologic aspects, seem to be on the threshhold of rapid advance contributed by clinicians who had not been hepatologists. Transplantation is one advance. This editorial points out that clinical genetics has made a contribution to a field overtly not related to genetics. This contribution, however, may clarify the etiology of one of the main diseases of hepatology which until now rested on empiricism in microbiologic, pathologic and clinical aspects.

Hans Popper, M.D., Ph.D.

Implications Of Enzyme Induction In Therapeutics

Titus Owolabi, 7T1

In clinical practice, individual variability in the rates of metabolism often markedly influences the therapeutic action of drugs. Metabolism of a given dose of a drug is sometimes so rapid in some patients that therapeutically effective blood and tissue levels are not attained; in others, the process may be so slow that drug toxicity results. This makes it difficult to predict the clinical response to a given dose of many drugs in an individual patient, especially one that the physician attends for the first time. Another difficulty is that *combined* drug therapy often results in toxicity, whereas at other times one drug may reduce the therapeutic efficacy of another. Thus salicylates inhibit the uricosuric effect of probenecid. Sympathomimetics induce a hypertensive crisis in some patients on MAO inhibitors. Thiazide diuretics, because of their hypo-potassaemic effect, often precipitate cardiac arrythmias in patients on digitalis. Such examples lend themselves to explanation on the principle of physiological agonists and antagonists.

A different kind of drug interaction, whose nature is only currently being understood, is the phenomenon of induction of drug-metabolising enzymes. The chronic administration of one drug sometimes reduces its own pharmacological activity as well as that of a totally unrelated drug. This is done by causing an increase in the amount of drug metabolising enzymes synthesized in the liver cell microsomes. Enzyme induction is a general phenomenon occuring in experimental animals, and in clinical practice. The principle has been evoked to explain lack of response in therapy or the development of drug tolerance following chronic administration of meprobamate, gluthethimide, tolbutamide and several other drugs. The list of drugs capable of acting as inducers is endless. It includes such ubiquitous agents as barbiturates, anaelgesics, tranquilizers, antihistaminics, hypoglycaemic drugs and uricosuric agents (Table 1). Recently cigarette smoking was reported to enhance nicotine metabolism in man. This may explain nicotine tolerance in smokers. The significance of this finding lies in the fact that nicotine and polycyclic hydrocarbons are known to enhance the metabolism of some drugs in experimental animals[1], and diminish the pharmacological activity of such drugs. Whether this is true in clinical practice is yet to be investigated.

Table 1, which is by no means complete, illustrates that most of the widely used drugs today are capable of inducing drug-metabolising enzymes, with the attendant therapeutic ramifications. These drugs are often the substrates of the enzymes in question, but the pharmacological consequences of the induction response may cause an altered response in unrelated drugs. In combined drug therapy, such altered response may be a consequence not only of induction but also of repression or inhibition of specific enzymes. One may therefore encounter potentiation as well as diminuition of drug activity in combined therapy.

TABLE 1:
DRUGS KNOWN TO ACT AS ENZYME INDUCERS

Hypnotics and Sedatives:	Barbiturates, Ethanol, Paraldehyde
Anaesthetics	Halothane, Ether, Nitrous Oxide,
CNS Stimulants	Amphetamine, Bemegride, Metrazol
Anti-convulsants:	Diphenylhydantoin (Dilantin) Paramethadione (Paradione) Trimethadione (Tridione)
Tranquilizers:	Meprobamate, Chlordiazepoxide (Librium) Phenaglycodol (Ultran)
Hypoglycaemics:	Tolbutamide (Orinase) Carbutamide Sulfanilamide
Antipsychotics:	Chlorpromazine Promazine Trifluprom azine
Muscle Relaxants:	Orphenadrine Zoxazolamine Carisoprodol
Anti-Inflamatory:	Phenylbutazone
Analgaesics:	Aspirin, Morphine, Aminopyrine
Antihistaminics:	Chlorcyclizine, Diphenhydramine
Alkaloids:	Nicotine, Cotinine
Insecticides:	Chlordane, D.D.T., Pyrethrums
Steroid Hormones:	Testosterone, Cortisone, Estradiol

Abridged from Conney, A. H.[2]

PHENOBARBITAL

Administration of phenobarbital lowers the plasma level of bishydroxycoumadin in man, and decreases its anticoagulant effect. This is in agreement with the work on experimental animals in which pretreatment of rats with phenobarbital for several days increases the activity of the liver microsomal enzyme which metabolises dicumarol and related anticoagulants.[3] Conney and co-workers[4] showed that simultaneous administration of both drugs can totally abolish the anticoagulant effect of dicumarol. Discontinuation of phenobarbital administration led to excessive bleeding. Similar observations have been reported by other investigators who noted that the route and timing of dosage significantly influence this barbiturate effect.

Using sixteen volunteers, Busfield *et al* showed that prior administration of phenobarbital consistently lowers the plasma level of the antifungal, griseofluvin.[6] The relevance of this finding to griseofluvin therapy cannot

be assessed from their report, nor can the mechanism of the phenobarbital effect.

Phenobarbital stimulates the inactivation of other clinically useful drugs. Plasma levels of diphenylhydantoin (Dilantin) were found to be persistently lower in a group of patients on combined Phenobarbital-Dilantin therapy than in another group on Dilantin alone, although both groups received 300 mg. of Dilantin daily. The interaction between these two groups poses no real problem in the management of epilepsy since phenobarbital also has anticonvulsant activity. A problem does exist however when an epileptic is put on Dilantin and another drug with enzyme inducing properties but no anticonvulsant activity; for example, tolbutamide in the case of an epileptic patient who is also an adult diabetic. A similar problem is posed by a combined therapeutic approach involving the antiinflammatory drug phenylbutazone (Butazolidine) and aminopyrine, an analgesic and antipyretic. The plasma levels and therapeutic effectiveness of aminopyrine are found to be greatly reduced[7].

From the foregoing, one aspect of enzyme induction is obvious, *viz* how the process can complicate drug therapy. There is, however, a more positive aspect: the principle can be used for specific therapeutic purposes. This will become obvious after a discussion of the mechanism of the process, in the light of current thinking.

MECHANISM OF ENZYME INDUCTION

Drugs capable of inducing enzymes that metabolise these and other drugs have heterogeneous chemical structures. A single molecular basis of enzyme induction is not known. However, observations reported by current investigators in the field have provided a working hypothesis for the molecular model of enzyme induction. The process occurs only *in vivo*. It may be blocked by actinomycin D and mitomycin C, both of which block nucleic acid synthesis, and by puromycin and cyclohexamide, which block protein synthesis at the ribosomal level[8]. Animal experimentation shows that inducer-drugs cause a marked increase in the smooth endoplastic reticulum of liver cells as observed in electron microscopy.[9] Histochemical studies reveal that the drug - metabolising enzymes are associated with the "smooth-surfaced" microsomes. All these observations cummulate in the conclusion that induction is a *de novo* synthesis of enzymes which inactivate some drugs.

Remmer and Merker[9] also reported that injection of phenobarbital causes an increase in the liver microsome enzymes which inactivate chloramphenicol, hexobarbital and aminopyrine. They suggested that this fact explains the clinical observations that drug tolerance often follows chronic administration of barbiturates.

Sladeck and Mannering[10] most recently postulated the existence of two groups of inducers. The first, typified by phenobarbital, consists of non-specific enzyme inducers that will cause inactivation of a wide variety of drugs. The second group will induce the degradation of only a narrow spectrum of drugs. This latter group consists mostly of polycyclic hydrocarbons. Both groups induce drug metabolism via a *de novo* synthesis of enzymes. One explanation offered is that barbiturate-like inducers stimulate the transcription stage of protein synthesis, i.e., speed up DNA—> mRNA stage. A hydrocarbon-type inducer speeds up the translational mRNA—> tRNA stage (Fig. 1). By either process, enzyme synthesis is increased. Another possible explanation is that the phenobarbital group increases the synthesis of a unique mRNA which causes accelerated synthesis of enzymes. The enzymes synthesized are those responsible for dehalogenation, de-esterification and various reductive and conjugative transformations of drugs. These are key steps in drug inactivation, the control of which strategically modify drug metabolism.

In support of their second explanation, these two investigators recently produced evidence for the *in vovo* synthesis of two qualitatively different types of Cytochrome P 450, a mixed function oxidase which acts as an electron acceptor in drug oxidation by liver microsome enzymes[11]. This suggests a strong possibility for two different types of enzymes, one for each group of inducers.

How inducers stimulate transcription or translation is unknown. Nor is it yet known why certain drugs are better inducers than others while some others have no such activity at all. The lack of detailed knowledge on the molecular mechanism of enzyme induction has not prevented its utilization in evaluating drugs, and in some controlled clinical trials. These will be considered presently.

ENZYME INDUCTION AND CLINICAL PHARMACOLOGY

Enzyme Induction can seriously affect the results of tests meant to evaluate new drugs or to evaluate drug metabolism when patients are in acute or chronic drug toxicity. New drugs are tested for effects on the foetus by feeding sub-toxic doses to male and female rats for a preliminary period, usually two months, and then through two pregnancy cycles.[2] This procedure is intended to detect any adverse effect of the drug on fertility, spermatogenesis, implantation and teratogenesis. If the drug under study stimulates its own metabolism, an erroneous interpretation of the results could be made.

Chlorcyclizine, an antihistaminic, when administered to pregnant rats during days 10 to 15 of gestation, produced a high incidence of cleft palate in the offspring. If administered from days 1 to 15 of gestation foetal concentrations are low because the relatively chronic administration stimulated the synthesis of enzymes which generally metabolized the drug. No cleft palates occured in the offspring. The latter procedure would sug-

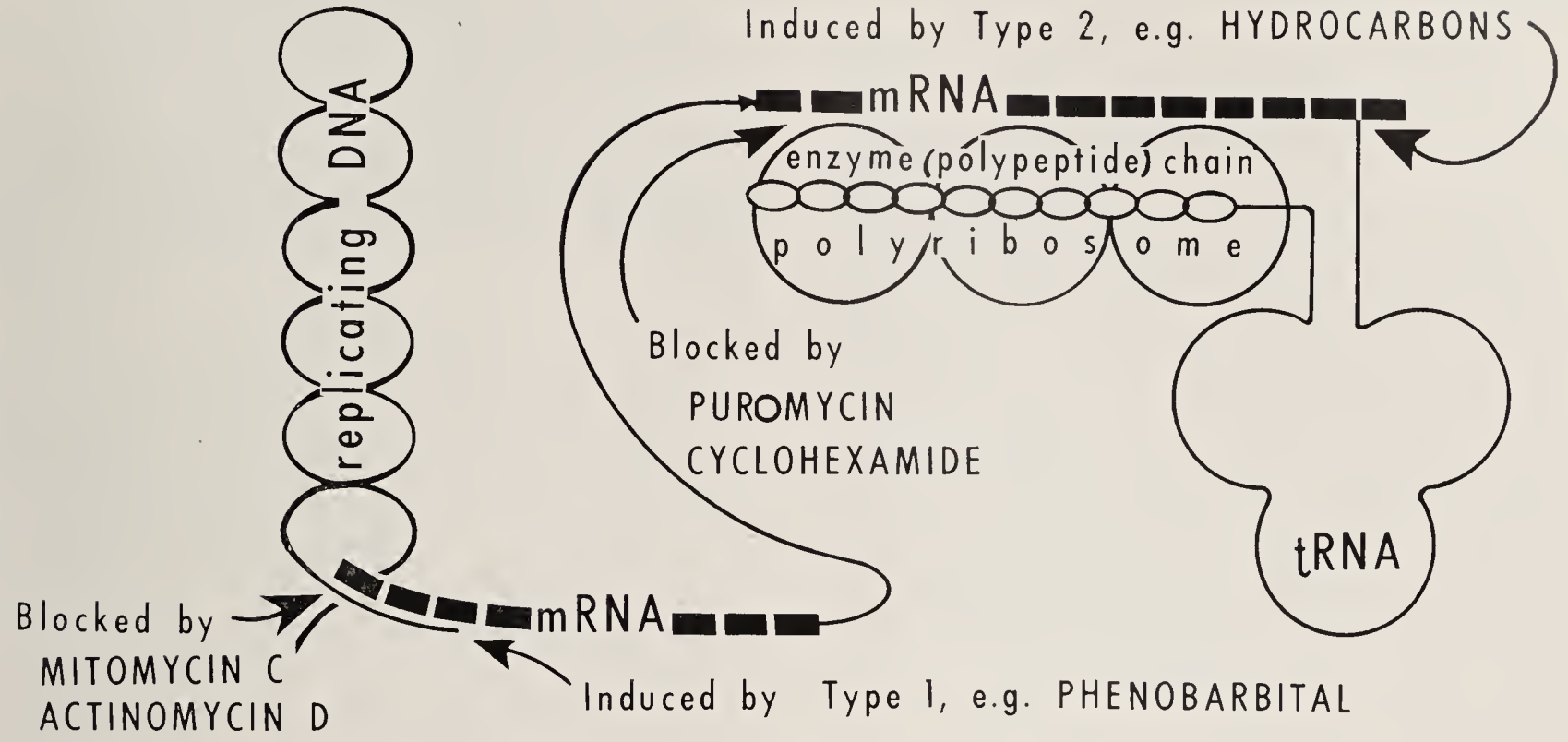

Fig. 1. Enzyme Synthesis (Schematic): Sites of Action of Blockers and Inducers.

gest a false conclusion that the drug has no teratogenic effects, unless the possibility of a process like enzyme induction was kept in mind in evaluating the results.

CLINICAL APPLICATION

Since Brown and co-workers first showed[12] in 1954 that mice fed the carcinogen 3-methyl-cholanthrene developed increased ability to demethylate another carcinogen, 3-methyl 4-dimethyl amino azobenzine and to convert it to non-carcinogenic products, the investigation of enzyme induction has added a significant potential to the chemotherapeutic approach to the management of "genetic" and other diseases. These include inborn errors of metabolism, endocrine disorders and the hereditary porphyrias of man.

INBORN ERRORS OF METABOLISM

Hereditary enzyme defects include galactosemia, phenylketonuria, glycogen storage disease and congenital non-haemolytic jaundice. Molecular bases for defective enzyme-forming systems of patients with inborn errors of metabolism are currently a subject of active investigation in many centers. Where low enzyme activity is due to a defective DNA molecule, enzyme induction is unlikely to be a successful therapeutic approach. If on the other hand, the defect is due to an abnormal DNA *regulation* of enzyme synthesis, suitable enzyme inducers may serve as a good therapeutic approach. Examples of these are galactosemic patients treated with progesterone which enhances the metabolism of galactose, and patients with glycogen storage disease treated with triamcinolone which stimulates hepatic glucose-6-phophatase activity.

HYPERBILIRUBINAEMIA

Yaffe *et al* claimed the first deliberate therapeutic application of enzyme induction in 1966 when they administered phenobarbital and observed a reversion to normal in a hyperbilirubinaemic infant.[13] Their rationale was stragihtforward. The child was entirely normal except for the presence of icterus. Pharmacokinetic studies with salicylamide revealed a congenital lack of glucuronyl transferase (Crigler-Najjar syndrome). Phenobarbital is known to induce formation of this enzyme which is necessary for bilirubin conjugation. Treatment with phenobarbital enhanced glucuronide-conjunction in the liver and lowered serum bilirubin. Jaundice cleared up until the therapy was discontinued. Re-institution of the same therapy at 15 months of age again lowered serum bilirubin and cleared the jaundice. Kreek and Sleisenger[14] also reported using phenobarbital to treat successfully a case of adult congenital non-haemolytic, unconjugated hyperbilirubinaemia.

ENDOCRINE DISORDERS

Induction of liver microsome enzymes may be of use in the management of metabolic diseases due to non-tumorous over-production of hormones. It has been shown that diphenylhydantoin stimulates production of 6 βeta-hydroxylase, an enzyme which de-activates cortisol. Werk *et al* gave the drug chronically to two patients with non-tumorous Cushing's syndrome and observed clinical and biochemical remission.[2] Similarly, administration of *o, p'*-DDD, an antitumour drug (isomer of the insecticide DDT) resulted in clinical and biochemical remission in Cushing's syndrome before any effect of the drug on the rate of cortisol excretion is observed, suggesting an induction mechanism.

HEREDITARY PORPHYRIAS

A corollary to induction is the observation that certain drugs inhibit the activity or synthesis of drug-inactivating enzymes. The mitochrondial enzyme δ-Aminolevulinic acid synthetase controls the rate of porphyrin and heme synthesis in the liver. Its induction may be controlled by both genetic and exogenous factors.[8] The enzyme activity is low in the normal liver but may be greatly increased by many drugs, steroids and foreign chemicals, including substances known to provoke an exacerbation in the hereditary porphyrias of man. It has therefore been suggested that the primary effect of porphyria-genic substances is the stimulation of enhanced production of the specific m-RNA for δ-ALA synthetase. The implication is that in the management of hereditary porphyria, drugs which induce δ-ALA synthetase are contraindicated. These include most steroids. The search currently is for a drug that will specifically inhibit δ-ALA synthetase.

ANTICARCINOGENESIS

Certain inducer drugs when given to experimental animals, stimulate enzymatic detoxification of several chemicals known to be carcinogenic. The formation of cancer by several of these carcinogens (aminoazo dyes, 3-4 benzypyrene, etc.) is blocked in rats treated with a variety of inducers. The observation suggests the theoretical possibility of using suitable inducers prophylactically to protect people from environmental carcinogens.

Finally it has been reported[4] that phenobarbital administration has a nonspecific anabolic effect on rat liver. Bile flow is increased, liver function and the metabolism of a variety of normal body substrates similarly increased. The generalized effect of phenobarbital on liver growth and function has aroused the speculation that it may be useful in the treatment of some forms of liver diseases. The speculation is further increased by the observation that little or no microsomal hydroxylase activity is detected in the newborn and regenerating liver, and in a variety of hepatomas.

The amount of knowledge at hand on the process of enzyme induction has opened up more avenues in the therapeutic approach to a number of diseases. It seems reasonable to suggest that as better understanding is achieved in the field, more and more applications will be made. The physician may then be able to achieve radical cure in the management of a variety of diseases for which the current "treatment" is a palliative relief of symptomatology.

SUMMARY

Enzyme induction is the process by which drugs and other chemical substances act to accelerate their own metabolism and thereby diminish the therapeutic effects. The molecular details of this process are not fully known at present. What information is available is however adequate to explain the development of tolerance in the chronic administration of some drugs, and to explain drug toxicity, potentiation or dimunition of drug action in some cases of combined drug therapy. Enzyme induction has had some clinical applications. A better understanding of the process looks promising for the management of disease due to enzyme deficiencies.

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Alcohol And The Liver

George Ebers 7T0

Heavy use of Alcohol is associated with at least three clinicopathologic entities related to the liver, viz., fatty liver, acute alcoholic hepatitis and cirrhosis. This article will deal with the pathogenesis of these sequelae of alcoholism.

There has been an increasing morbidity and mortality due to alcoholic fatty liver and cirrhosis in the past few decades. The death rate in the U.S.A. from cirrhosis has increased between 1930 and 1960 from 7/100,000 to 14/100,000, in white males. Surprisingly cirrhosis of the liver was the leading cause of sudden and unexpected natural death in adults aged 20 to 39 years in a study in a large American city.[1]

The association of alcoholism and cirrhosis has long been recognized, cirrhosis being about seven times more frequent in alcoholics than in the general population. However, it is interesting to note that only one in twelve of severe inebriates go on to develop cirrhosis. The reason for this is still a mystery. Genetic susceptibility may be a factor.

ETHANOL AS A HEPATOTOXIC DRUG

Considerable work has been done in the past few years to determine whether or not ethanol is directly toxic to the liver. A summary of the findings follows.

Liver injury produced by ethanol has been reported to be dose-related, reproducible in man and experimental animals and is characterized by fatty metamorphosis and necrosis.[2] It is theorized to be a direct effect comparable to carbon tetrachloride, as opposed to the hypersensitivity type of injury produced by other agents. However, in rats given ethanol, acutely or on a chronic basis there appears to be no impairment of lipoprotein production or release as there is with carbon tetrachloride. The result depends on ethanol dose or concentration. Lieber et al believe that such impairment is not an important factor in the production of fatty liver.

Ultrastructurally there is increased smooth endoplasmic reticulum accompanying ethanol administration both to man and animals but this increase is doubled when the diet is deficient in protein and lipotropes. In mammalian liver the functions of smooth endoplasmic reticulum are thought to include facilitation of excretion of highly lipid soluble compounds by converting them to more water-soluble derivatives. The increase in smooth endoplasmic reticulum is thought to be an adaptive change to lipid accumulation caused by alcohol enabling facilitated conversion of water insoluble lipid to water-soluble lipoproteins (the mechanism of lipid accumulation will be dealt with later). Marked structural alterations of mitochondria occur after ethanol and the function of these organelles may also be impaired as evidenced by decreased capacity to carry out oxidation of various substrates[3] (the Mallory body seen in alcoholic cirrhosis is a megamitochondrion).

There is the possibility that some of the congeners of alcoholic beverages may play hepatotoxic roles. For example, the wine of certain vintages has been shown to contain large amounts of iron which was shown to accumulate in the liver and other organs of experimental animals given wine over long periods. Although wines and beer were once believed to be most harmful to the liver it has been shown that whiskies are equally liable to lead to cirrhosis. It has also been said that beer is less likely to cause cirrhosis because it contains protein and vitamin B, but the content of protein is only about 3 Gm. per liter and niacin is the only B vitamin present in significant amounts.[4]

PATHOGENESIS OF ALCOHOLIC FATTY LIVER

Fatty liver probably can be produced by alcohol even in the absence of dietary deficiencies. This has been shown in alcoholic volunteers whose hepatic morphology had returned to normal who were given diets more than adequate in protein content.[5] Liver biopsy with evaluation of hepatic lipid content showed this after isocaloric substitution of ethanol for carbohydrate. Isocaloric substitution of fat for carbohydrate produced a lesser degree of steatosis implying that ethanol is more steatogenic than fat itself. However, the subjects used in this study had been alcoholics. Their nutritional history may have conditioned their response to ethanol. Similar work has been done in rats and different investigators have reported fairly similar findings with opposite interpretations and conclusions. Studies have shown that a decrease in dietary fat does not prevent ethanol induced steatosis although it does significantly reduce it; as does replacement of dietary lipid with medium chain triglyceride.

Lipotropic factors (viz. choline, methionine) do play an important role in fatty liver and even cirrhosis in rats but this is probably not true in the human.

Lipids accumulating in the liver may come from three sources:

1) dietary lipid
2) adipose tissue
3) lipids synthesized in the liver

Fatty acids deposited in the liver after prolonged ethanol intake with a normal fat-containing diet are largely derived from dietary lipid as discussed below.

MECHANISMS OF LIPID ACCUMULATION

1) Increased peripheral fat mobilization

The triglyceride content of fatty livers in rat and man appears to be different from that of adipose tissue. This

is probably not an important mechanism in the steatosis associated with chronic alcoholic intake.

Nevertheless in the acute fatty liver this peripheral mobilization may be important. Evidence on this seems conflicting but the result actually depends on whether the subject is fed or fasted.

2) Decreased hepatic lipoprotein formation and or release

Here again the findings are not clear-cut. Fatty liver develops with ethanol intake in vivo with no change or even increase in plasma lipid, especially triglycerides. If one ascribes a minor role to peripheral mobilization of fat in hepatic steatogenesis this would tend to speak against this mechanism of fatty liver production.[2] If lipoproteins were impaired in their production or release, the serum lipids would be expected to fall.) The pathogenesis of alcoholic hyperlipemia has not been worked out but (in the rare Ziêve's syndrome) decreased lipolysis may play a role. In these subjects there is evidence of low lipoprotein lipase activity and of a circulating inhibitor of this enzyme. Further experiments in human volunteers have shown that alcoholic hyperlipemia occurs in the first ten days following a 300 gm per day ethanol intake but that after this time serum lipids return to normal. This indicates that the duration of alcohol intake determines whether or not hyperlipemia is seen.

3) Decreased lipid oxidation

Ethanol metabolism results in the increased formation of NADH as it is broken down to acetaldehyde via alcohol dehydrogenase. This may result in decreased citric acid cycle activity and decreased lipid oxidation with accumulation in the liver of lipid. In fact, in studies on this subject the other parameters of the handling of dietary lipid were found to be unaffected by ethanol, i.e. lipid absorption, chylomicron disappearance from the plasma, and hepatic chylomicron uptake. Since medium chain triglycerides are more easily oxidized by hepatic microsomes than long chain fatty acids it might explain why their substitution for dietary fat reduces hepatic lipid accumulation after prolonged ethanol intake.

4) Increased hepatic lipid synthesis —

Ethanol oxidation results in production of acetyl CoA, acetate and $NADH_2$. $NADH_2$ is generated by the oxidation of several substrates such as glucose. However, unlike glucose, ethanol metabolism is selectively performed in the liver. Thus there is an increase in the $NADH_2$ ratio in the cytoplasmic compartment where the metabolism of ethanol occurs.

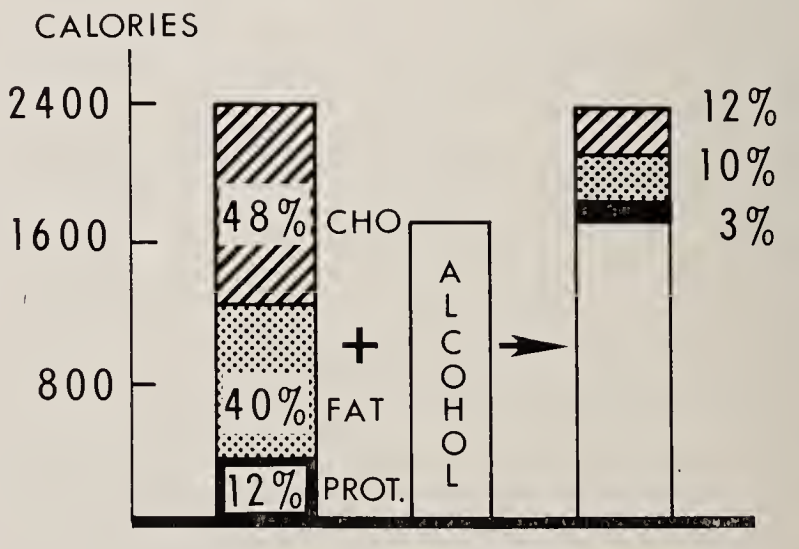
It appears that the mitochondrial membrane is rather impermeable to $NADH_2$, so that if the mitochondrial flavoprotein-cytochrome system has to take part in the re-oxidation of $NADH_2$ produced in the soluble compartment, it is likely that some intermediate such as alpha-glycero-phosphate transports hydrogen from one compartment to the other. Alcohol oxidation has been found to be accompanied by an increase in hepatic alpha-glycero-phosphate which could contribute to the accumulation of hepatic esterified lipids through enhanced production of their glycerol moiety. Perhaps enhanced hepatic lipogenesis can be considered as a means for the hepatic cell to dispose of the excess hydrogen produced on ethanol oxidation. The enhanced cholesterol genesis which has been reported to be produced by ethanol might be explained on the same basis using up the excess hydrogen by-product of ethanol oxidation. The derangement in $NADH_2/NAD$ ratio has also been used to account for other metabolic derangements seen in alcoholism, i.e. alcoholic hypoglycemia.

One other mechanism should be included in the discussion of the pathogenesis of fatty liver. Di Luzio et al[8] have shown the development of lipid peroxides in acute ethanol toxicity and have suggested the following sequence:

1) ethanol + NEFA + 'O' $\longrightarrow$
2) formation of lipoperoxides $\longrightarrow$
3) peroxidative degradation of structural membrane lipids $\longrightarrow$
4) enzymatic, ultrastructural and permeability alterations $\longrightarrow$
5) fatty infiltration and/or necrosis.

Other investigations have failed to show lipid peroxidation in the liver in acute ethanol toxicity.

Perhaps, hepatotoxicity of ethanol is not very pertinent to the case of the true life alcoholic. The chronic alcoholic almost necessarily presents a mixed picture of excess alcohol ingestion and protein deficiency as Hartcroft points out. In a sedentary 70 kg man, the caloric requirement is about 2400 calories per day. If he consumes one pint of whisky per day, 1800 calories are provided. This leaves 600 calories to be provided as solid food. The recommended dietary allowances of fat, carbohydrate and protein are seen in the bargram below:



ADAPTED FROM LEIBER C.S.[7]

The effect of addition of 1800 calories per day of alcohol is seen to the right of this bargram. To maintain adequate nutrition it is obvious that a tremendous surplus of calories is necessary, which would cause a weight gain of 1 lb. per two days if the excess calories were efficiently utilized. From this discussion it is seen that the alcoholic almost invariably presents his liver with the dual insult of ethanol toxicity and rather severe dietary deficiency. In fact, some investigations in the rat have shown no difference in the development of fatty liver between those on a deficient diet and those on a deficient diet given ethanol.[6]

RELATIONSHIP OF FATTY LIVER TO CIRRHOSIS AND ALCOHOLIC HEPATITIS

Some believe fat accumulation in the liver is harmless while others believe it to be a direct precursor of cirrhosis. Transition of fatty liver to cirrhosis has been documented but there is no evidence that the development of alcoholic fatty liver is directly responsible for cirrhosis. Although fat deposition almost invariably follows excess alcohol consumption, cirrhosis does not develop in all alcoholics. It is therefore obvious that moderate or intermittent fatty liver does not necessarily lead to cirrhosis.

Clinically, alcoholic fatty liver appears to be a rather reversible and benign condition. In some cases however, usually following a period of intensified drinking by a chronic alcoholic, a more severe clinical state develops which has been termed "alcoholic hepatitis". It is characterized by anorexia, nausea, vomiting, upper abdominal pain, hepatomegaly and frequent fever and jaundice. Biochemical tests may show liver cell damage and occasionally an obstructive element. Anemia is usually present and severe leukocytosis may develop. In addition to marked fatty changes the histologic picture is characterized by local and massive cellular degeneration and necrosis with acute inflammatory reaction. (This picture is separable from fatty liver by light microscopy.) E M studies show much enlarged and clumped mitochondria. There is vesiculated endoplasmic reticulum, a paucity of parallel arrays of rough E R and extensive areas of cytoplasmic degradation. Similar changes may be seen in fatty liver which suggests fatty liver may be a precursor of alcoholic hepatitis. The extensive inflammation and degeneration seen in alcoholic hepatitis would seem more likely to result in the fibrosis of cirrhosis but such a chain of events is hypothetical.

In addition to dose and duration of alcohol consumption factors such as malnutrition, pattern of alcohol intake, genetic and constitutional predisposition and possibly congeners of alcoholic beverages may play varying roles of importance in the development of cirrhosis.[10]

SUMMARY

Alcoholism is associated with fatty liver, alcoholic hepatitis and cirrhosis. There is some evidence that ethanol is toxic to the liver and causes fatty liver possibly by decreasing lipid oxidation and enhancing hepatic lipogenesis. The source of the fat is largely derived from dietary lipid. The true life alcoholic presents a mixed picture of dietary deficiency and ethanol toxicity, both of which contribute to fatty liver. The relationship of fatty liver to subsequent developmentary cirrhosis is unclear. Alcoholic hepatitis may be a precursor of cirrhosis. There is a lack of long term studies on alcoholics who go on to develop cirrhosis.

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Viral Hepatitis

D. A. Ruggiero, B.S.c. (Med.) 7T0

Epidemics of infectious jaundice have been recognized for centuries; as long ago as 1885 physicians recognized jaundice following vaccination. The concept that an "infectious, filterable agent" (virus) may have been the cause of jaundice evolved from results obtained from World War II soldiers (volunteers) who had been fed Seitz filtrate of stools from patients with acute cases of hepatitis and who developed hepatitis approximately one month later.[1]

Current views are that viral hepatitis is an acute systemic infection with characteristic pathologic liver lesions caused by more than one virus. There are two categories of the disease: infectious hepatitis (I.H., epidemic hepatitis, virus A. hepatitis), and homologous serum hepatitis (S.H., post-transfusion, virus B. hepatitis).

ETIOLOGY

In spite of persistent efforts by many virologists, the agents for either I.H. or S.H. have not been identified with certainty; adequate virological and serological experimental systems are still lacking at present. Some serologic and some epidemiologic studies have suggested that both I.H. and S.H. are caused by immunologically distant viruses. However, other well documented results have suggested that both I.H. and S.H. may be the result of infection with the same virus.[2]

More recently Blumber, B. S. et al, and Sutnick et al, have detected Australia antigen in acute and chronic viral hepatitis and in carriers who have transmitted hepatitis to recipients of their blood[3 4 5]

Millman et al, have shown that a specific fluorescein-tagged antibody to Au(1) reacts with an antigen located in or on the surface of the nuclei of hepatocytes from patients with viral hepatitis. At present this group is evaluating the use of their "A(u) Fluorescent Test" as a specific means of diagnosing viral hepatitis.[6]

EPIDEMIOLOGY

(a) *I.H.*

This form of viral hepatitis occurs both sporadically and in epidemic forms. The latter is seen particularly under conditions of poor sanitation and overcrowding. Explosive epidemics may follow exposure to feces-contaminated water or food (clams and oysters).

(b) *S.H.*

This virus infection gains entry to the body by the parenteral route; as little as 0.00004 ml. of infectious serum can produce the disease.[7] This amount may be introduced in a wide variety of ways: therapeutic administration of blood, contaminated needles and syringes, and dental instruments. The use of drugs, principally amphetamines, self-administered parenterally (mainlining) by young people with contaminated needles and syringes has led to an epidemic rise in this form of hepatitis.

The principal differences between the two viruses are summarized in table 1, which is based on the W.H.O. Expert Committee on Hepatitis (1964).

TABLE 1

	I.H.	S.H.
Incubation period (days)	15 - 50	50 - 180
Age preference	children young adults	any age
Route of Infection	1. fecal-oral 2. parenteral	parenteral
Virus in feces	2 wks. before jaund'ce to 2 wks. after jaund'ce	absent
Virus in blood	same as for feces	80 - 90 days before to 10 days after jaundice

CLINICAL TYPES

(1) Acute icteric hepatitis (a) Typical
(b) Cholestatic
(2) Acute anicteric hepatitis
(3) Fulminant hepatitis
(4) Subacute fulminant hepatitis
(5) Relapsing hepatitis
(6) Post-hepatitis cirrhosis
(7) Chronic hepatitis
(8) Post-hepatitis syndrome

Only the first three types will be briefly discussed.

1. Acute Icteric Hepatitis

(a) *Typical*

This type occurs in 80% of I.H. and 20% of S.H. In most patients there is a *pre-icteric* stage lasting from 3 - 8 days consisting of gastrointestinal "flu-like" syndrome consisting of constant anorexia, nausea, vomiting and low grade fever. Often these people develop a sudden distaste for cigarettes and alcohol. Towards the end of this stage the liver enlarges and becomes tender.

The *icteric* stage follows within a few days to one week; the urine is dark (conjugated bilirubin), the patient's sclerae are jaundiced and the temperature returns to normal.

The convalescent stage begins about 3 - 10 days after the onset of jaundice.

LABORATORY FINDINGS

1. Urine
   (1) in pre-icteric stage, bilirubin in the urine is the earliest manifestation;
   (2) in the pre-icteric stage, urobilinogen may also be detected in the urine;
   (3) at the height of the icteric stage very little bilirubin reaches the intestine; therefore urobilinogen disappears from the urine;
   (4) urobilinogen reappears again in the convalescent stage. It is an excellent indication of the patient's recovery.

2. Feces
   (1) The onset of clinical jaundice is marked by lightening of stool colour.
   (2) During the convalescent stage the normal colour reappears.

3. Blood
   (1) There is an increase in total and direct bilirubin.
   (2) S.G.P.T. and S.G.O.T. (serum glutamic pyruvic transaminase, and serum glutamic oxalo acetic transaminase) values are elevated with the peak level found 1 - 2 days before or after the onset of jaundice. Later in the course the level of these enzymes falls even if the patient is clinically worsening. Therefore their estimation cannot be used prognostically.
   (3) The prothrombin time may be increased and does not return to normal with vitamin K therapy.
   (4) The E.S.R. is high in pre-icteric stage, falls to normal with development of jaundice, and rises again when jaundice subsides.

(b) *Cholestatic*

Acute hepatitis is considered cholestatic when jaundice is unusually deep and protracted. These patients usually present with elevated serum alkaline phosphatase, pruritus, and a ratio of S.G.O.T./S.G.P.T. less than 1.0 reflecting hepatocellular disease. It has been postulated that the inflammatory reaction involves the peritubular cholangioles allowing the regurgitation of bile.[8]

2. Acute Anicteric Hepatitis

Some authorities claim[8] that I.H. occurs as a subclinical disease in probably 80 - 90% of cases. In recognized cases the syndrome is fairly well defined; liver function tests may show some pattern as seen in acute icteric hepatitis but often changes are less striking. A palpable enlarged tender liver is found in 80% of cases. The high incidence of immunity to I.H. virus infection in adults with no history of hepatitis supports the view that anicteric hepatitis is probably a common disorder.

3. Fulminant Hepatitis

This form is usually fatal within 10 - 14 days. Occasionally the course is so extremely rapid that death occurs before jaundice appears. Death occurs in less than 0.5% of I.H. and in 10 - 12% of S.H.

The pre-icteric symptoms are more severe and jaundice is deeper than in acute icteric. Terminally these patients lapse into deep coma and may present with convulsions, shock, oliguria and azotemia.[9]

Prior to the development of coma the patient usually shows low prothrombin level, low plasma fibrinogen, and low serum albumin.

DIFFERENTIAL DIAGNOSIS

(a) In the *pre-icteric* stage:
   (1) Infectious mononucleosis
   (2) Acute appendicitis
   (3) Acute gastroenteritis

The presence of bilirubinuria, rise in S.G.P.T. and S.G.O.T., and tender enlargement of the liver, help rule out (2) and (3). A positive Paul Bunnell test, lymphadenopathy and atypical lymphocytes would point to infectious mononucleosis.

(b) In the *icteric stage* one must differentiate acute hepatitis from obstructive jaundice. The following liver function tests are useful in the differentiation. (Table 2)

TABLE 2

Test	Viral Hepatitis	Obstructive Jaundice
Alkaline phosphatase K.A. units	<30	>30
S.G.O.T.	>300	<300
S.G.P.T.	>400	<300
Se Cholesterol	Normal or ↓	>300 mg %
Prothrombin time	if ↑ Vit. K has no effect	if ↑ Vit. K corrects

TREATMENT

(a) *Prevention*

Secondary spread of I.H. can be controlled by instructing the patient in general hygiene, safe disposal of feces and by advising him to follow meticulous toilet habits.

The experimental work of Krugman et al[10] in 1962 suggested that the majority of patients were no longer infectious by the time jaundice was at its peak. Most research data indicates that both stool and blood are maximally infectious in the pre-icteric stage.

Numerous studies have established the efficacy of gamma globulin in modifying I.H. and in many cases prevention of clinical infectious hepatitis.[8]

The dose of 0.04 ml of gamma globulin per kilogram body weight should be administered to close contacts and to control epidemics; this should be given as soon as possible.[11] A dose of 0.12 ml/kg should be given every 6 months to people travelling to an area where hepatitis is common. There is uncertainty and confusion regarding the usefulness of gamma globulin in the prevention of serum hepatitis. Numerous studies present contradictory results.[12 13] At the present time one cannot enforce this method of treatment for serum hepatitis until further studies clarify the role of gamma globulin in the prophylaxis of serum hepatitis.

(b) *Clinical Treatment*

Since treatment appears to do little in altering the course of viral hepatitis, most programs for treatment are on an empirical basis. Management of hospitalised patients should include bed rest during the icteric stage and an adequate caloric intake. Liver function tests, especially serum bilirubin, S.G.P.T. and S.G.O.T., should be done at least once per week during the acute stage of the illness.[14]

After the bilirubin is less than 1.5 mg %, and urobilinogen has disappeared from the urine, the patient is allowed to convalesce. Dr. Sheila Sherlock recommends that the patient's period of convalescence should be two times the period spent in hospital or in bed at home.

Corticosteroids are not indicated in the usual case of hepatitis. They may be of value in the fulminant case, and hepatic coma or precoma associated with acute viral hepatitis. In these cases 500 - 1,000 mg of hydrocortisone intravenously per day is recommended.[15]

Subacute Fulminant Hepatitis

In this variant of viral hepatitis fluctuating jaundice, low grade fever, nausea and vomiting persist. The patient presents with hepatomegaly and splenomegaly. Clinically hepatocytic failure becomes evident; the patient shows spider naevi, ascites, hypotension and, terminally, precoma and coma. Blood biochemical tests also reflect hepatocytic failure.[9]

Prognosis is poor; death from liver failure occurs within 6 - 12 weeks of the onset. However, some clinical recoveries are noted, but hepatitic cirrhosis is inevitable.

Relapsing Hepatitis

In 5 - 15% of patients with viral hepatitis there are relapses. The relapse is manifested by similar biochemical abnormalities as in the original attack. Often the relapse is precipitated by "premature activity or by intake of alcohol."[9]

Post-hepatitis Cirrhosis

This is probably a very rare entity but viral hepatitis may be an important precursor to cirrhosis. The absence of a past history does not exclude hepatitis since anicteric attacks usually are not recorded. Serial hepatic biopsies give some support that acute viral hepatitis may progress through a subacute stage until it becomes cirrhosis. Clinically these patients present with hepatocytic inadequacy and either succumb or revert to an inactive cirrhosis.

Post-hepatitis Syndrome

After an attack of viral hepatitis some patients may develop certain symptoms, probably of psychological origin. Its prominent features are anxiety, fear of future jaundice, fatigue, failure to gain weight, anorexia with fat intolerance, and right upper quadrant discomfort.[9] These patients have no organic liver disease as demonstrated by normal biochemical tests of urine and serum.

[*A comprehensive review of Chronic Active Hepatitis is provided in the Clinicopathological Conference, page 135.—Ed.*]

CONCLUSION

Since at the present time there is no specific treatment for viral hepatitis, our effort should be directed toward the early diagnosis and prevention of secondary cases. A careful reconstruction of the epidemiologic history helps establish appropraite measures for preventing further transmission. Gamma globulin should be administered as soon as possible to contacts. Finally, cases should be reported to public health department in the community.

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Ascites

Judith E. Friesen 7T1

Ascites is defined as the accumulation of fluid in the peritoneal cavity. It may be serous, hemorrhagic, or chylous. It is important not only because it drains body protein when tapped, disturbs salt and water homeostatic mechanisms, and demands significant circulatory adjustments, but also because it indicates that the causative disease is far advanced.[1] To be clinically detectable in the adult, two liters must be present; the largest amount recorded is seventy liters.[2]

The symptoms experienced by the patient who has developed ascites are an expanding girth, a feeling of heaviness and distending pressure in the abdomen, increased flatulence, and, if the quantity is large, increasing shortness of breath on exertion and dyspnea at rest. On physical examination,[3] he is seldom in distress because of his ascites but may be seriously ill with liver or heart disease, or cancer or infection in the abdomen. In the supine position, the flanks bulge and the greater circumference is at or above the umbilicus. The umbilicus may be midline, flush to the surface or pushed out, and may be closer to the pubis than to the xiphoid. Abdominal veins may be dilated with blood running from the groin up towards the axilla, as seen when severe portal hypertension is present, or, rarely, out from the umbilicus in a caput Medusae.

If only a small amount of ascites is present, the abdomen feels soft and unguarded; if a large amount, increasingly tense. The heart apex is displaced laterally and upward. An enlarged liver or spleen may be impalpable beneath the fluid until a quick depression of the corresponding hypochondrium brings the organ up to the surface. A thrill carried by the fluid may be elicited while an assistant dampens the pulse transmitted by the abdominal wall. On percussion, the flanks are dull over the fluid and the central abdomen is tympanitic over the floating air-filled bowel. If the mesentery is held down by fibrous adhesions as in tuberculous peritonitis, or if the fluid is loculated as in chronic peritonitis, this clear pattern is not heard. Shifting of the dullness after the patient rolls to one side demonstrates the movement of the fluid to the most dependent region and is considered to be the cardinal sign of ascites. Bowel sounds are usually present.

Other conditions which appear similar to ascites but can usually be differentiated by history and physical examination are: 1) tympanites of pregnancy or a distended bladder. The flanks are tympanitic and girth is greatest below the umbilicus, 2) ovarian or abdominal cyst or hydronephrosis – the signs are lateralized; and 3) obesity – the flanks are tympanitic and the umbilicus is dimpled. The diagnosis of ascites is seldom difficult to make; however, treatment depends on knowledge of the etiology and pathogenesis of the condition.

PATHOGENESIS OF ASCITES

The pattern of distribution of extracellular fluid in the intravascular and extravascular compartments of the body depends on the hydrostatic and osmotic pressure forces in each, the former tending to drive fluid out of the compartment, the latter tending to draw it in. In the normal state, in which the volume of the compartments remain approximately constant, there exists an equilibrium such that

> Capillary hydrostatic pressure – Plasma oncotic pressure = Interstitial hydrostatic pressure – Interstitial oncotic pressure.

Simultaneous exchange between the compartments occurs continuously. If the balance of forces is altered, a net movement of fluid in one direction occurs until a new equilibrium is established. If it is from the intravascular to the extravascular, the clinical picture is that of edema and ascites; if it is from the extravascular to the intravascular, it is that of dehydration. Ascites, then, is the result of net movement of fluid into the peritoneal cavity following a disruption of the normal balance of forces in the abdomen.[1] Whether or not it is accompanied by edema depends on the presence and degree of imbalance of these forces elsewhere in the body.

PORTAL VENOUS HYPERTENSION

The most common cause of ascites in North America is liver disease, particularly portal cirrhosis associated with chronic alcoholism and malnutrition.[4] The fibrosis which develops around the portal areas, the hepatic veins, and finally between the two forming dense bands of connective tissue across the lobules destroys liver architecture. This leads to circulatory obstruction and rerouting of significant amounts of blood through single sinusoids. It has been observed that arterial blood under high pressure gains free flow into some sinusoids but it is not generally accepted that this pressure is transmitted back into the portal venous system.[5] Contributing to obstruction are regenerating nodules of parenchyma which easily grow against low pressure sinusoids, compressing them against stiff fibrous septa.

A hepatic outflow block develops which causes congestion in the liver, back-up and pooling of portal venous blood, and raised pressure in the splanchnic bed. As a result of this hypertension, the portal, mesenteric, and splenic veins become dilated and tortuous, the spleen becomes engorged with blood, and a great number of mesenteric and peritoneal arterio-venous and veno-venous shunts either open or develop carrying up to two-thirds of portal blood out of the sluggish portal system and into the Inferior Vena Caval and Superior Vena

Caval systems. The increased hydrostatic pressure in the portal capillaries and collateral network breaks the equilibrium resulting in transudation from a massive filtration surface into the peritoneal cavity. And in fact, at operation, the mesentery of a patient with portal hypertension is seen to weep fluid continuously.

Portal hypertension by itself rarely produces ascites. In a study of forty-six cases of extrahepatic portal vein obstruction,[6] twelve had a history of ascites. In eight of these the ascites was transient and was associated with a gastro-intestinal bleed or portal sepsis which likely reduced albumin levels. Four had permanent ascites with normal albumin. Factors other than portal hypertension also play major roles in ascites formation.

INCREASED HEPATIC LYMPH

Normally, hepatic lymph forms about half of thoracic duct lymph.[7] Ninety per cent is produced from blood in the sinusoids by transudation across endothelium into the spaces of Disse and is modified by water and salt exchange with the hepatic cells.[8] Because the endothelium is very permeable, this lymph has a protein content and oncotic pressure almost equivalent to that of blood; usually it lacks formed elements. It is added to in peribiliary lymphatics by a small amount (10%) of protein-poor lymph formed by transudation from the relatively impermeable hepatic artery and portal venule. It flows via a rich plexus of lymphatics in the fibrous perivascular capsule of Glisson to the porta hepatis and then reaches the venous system via the cysterna chyli and thoracic duct. Small amounts of capsular leakage into the abdominal cavity are picked up by peritoneal lymphatics and transported via the right thoracic duct and internal mammary lymphatics.

In cirrhosis, congestion and increased pressure in the sinusoids results in increased hepatic lymph formation in the spaces of Disse. The process may be enhanced following the action of vaso-active substances such as histamine which, experimentally, has been shown to increase endothelial permeability.[7] As well, anoxia and necrosis of endothelium may increase its permeability, especially to blood cells which are grossly apparent in thoracic duct lymph of cirrhotics.[8] As lymph formation increases, thoracic duct flow increases, from three to twenty times normal,[8] and eventually the system becomes overloaded. Lymphatic congestion and increased pressure ensue, and Glisson's capsule, perihilar, and liver surface lymphatics leak copious amounts of protein-rich fluid which cannot be carried away. The high oncotic pressure draws extracellular water out of surrounding abdominal tissue and into the cavity aggravating ascites.

In cirrhotic patients coming to post mortem, the greatly dilated hilar lymphatics commonly observed are taken to be evidence of this mechanism. Its plausibility was demonstrated in a classic experiment in which a dog's liver was placed above the diaphragm and the hepatic veins were tied off; the dog soon developed a hydrothorax due to lymphatic leakage.[6] In late cirrhosis, the protein content of ascitic fluid decreases.[8] This suggests that as the disease progresses, lymph is derived from a less permeable capillary bed and may be the result of capillarization of sinusoid endothelium – the laying down of collagen fibres and the formation of a continuous basement membrane under the endothelium – which would decrease protein movement.

It is generally thought that increased hepatic lymph does not initiate ascites formation but rather enhances and perpetuates that caused by portal hypertension.[1] Certainly obstruction of intestinal lymphatics prevents the transport of splanchnic bed transudate out of the abdominal cavity.

HYPOALBUMINEMIA

Plasma oncotic pressure depends primarily on its albumin concentration.[9] In cirrhosis, hypoxia and destruction of parenchymal cells results in decreased albumin synthesis. Hypoalbuminemia follows, the reduced plasma oncotic pressure disrupts the balance, and there is a net loss of water from the circulatory system to the interstitium and body cavities. Lymph loss to ascites carries albumin permanently out of the vascular system and renal salt and water retention tend to dilute its concentration further. Total serum protein weight may not be significantly reduced since there is often increased synthesis of gammaglobulin by circulating plasma cells.

Analbuminemia by itself produces generalized edema including some ascites. Hypoalbuminemia associated with portal hypertension, as in portal cirrhosis, localizes fluid loss to the peritoneal cavity and generalized edema develops late if at all.[11] That is, in the presence of portal hypertension, the hypoalbuminemia necessary to initiate ascites formation is less severe than that necessary to produce generalized edema. Dependent edema may develop when ascitic fluid compresses the I.V.C. significantly. This must be exceedingly rare. It is reversed by paracentesis although the edema fluid quickly accumulates in the abdominal cavity due to the influence of the unaltered portal hypertension.[5] Generalized edema may be reversed by albumin infusions restoring plasma levels to normal but this has little effect on the ascites.[5]

HORMONAL FACTORS

Body salt and water excretion and retention are controlled primarily by aldosterone and antidiuretic hormone acting on the distal tubules and collecting ducts of the kidney nephrons. In cirrhotics with ascites, for reasons that are not understood, salt and water are retained. Urine becomes scanty; sodium concentration in urine, feces, sweat, and saliva becomes very low.[11] One theory is that although increased pooling of blood in

the splanchnic bed results in increase in total blood volume, there is a relative decrease in blood in the arterial tree; that is, a decreased "effective" blood volume.[6] The subsequent reduced renal blood flow activates the renin-to-aldosterone system and excess salt is retained. High serum sodium levels then stimulate ADH elaboration and thirst with the resultant renal water retention and increased water intake. Damaged liver parenchymal cells may be unable to inactivate aldosterone and ADH at the normal rate holding their serum concentrations even higher. The retained salt and water go mainly into the ascitic pool, allowing continuation of the perverse mechanism; in time, total body water and salt become immensely increased.

This condition, then, is interpreted as secondary hyperaldosteronism. It is supported by observations of hypertrophic juxtaglomerular apparatus in kidneys of cirrhotic patients, high serum renin and aldosterone levels, and increased urine aldosterone excretion.[1] Treatment by multiple infusions of unmodified autogenous peritoneal fluid has been successful in at least temporarily reducing ascites, possibly because of its expansion of blood volume causing increased renal blood flow, and decreased aldosterone and ADH secretion.[1]

An alternative to the decreased renal blood flow theory has been suggested by Orloff who has demonstrated that in dogs, a minimal hepatic outflow block produces a hormone which acts via the kidney to stimulate aldosterone production.[9] However, measurements of the presence and activity of aldosterone in cirrhotics show baffling inconsistency. Some cirrhotic patients with or without ascites will retain significant amounts of sodium following a saline load; others will not. And there is not a good correlation between the sodium retained and aldosterone excreted.[1] Possibly aldosterone and ADH activity are unimportant, since, some think, the excessive sodium and water reabsorption is in the proximal tubule, due perhaps to decreased glomerular filtration or to lack of or inhibition of the postulated "third factor" (humoral natriuretic factor).[12] In this case, only small quantities of sodium and water would reach the distal tubule where the hormones begin to act. Some experiments[9] suggest that a significant decrease in renal blood flow, whether due to decreased arterial blood or to pressure from ascitic fluid on renal veins, does not exist consistently enough in cirrhotics with ascites to be a major etiological factor of sodium and water retention. Finally, Lieberman[13] demonstrated that total plasma volume did not rise in ten patients who had spontaneous loss of ascites, appearance of sodium in the urine, and increased GFR. Since portal hypertension remained constant, he assumed that splanchnic blood volume did not change and therefore "effective" blood volume was not different in the ascitic and non-ascitic phases. He concluded that sodium retention may be a direct result of splanchnic volume expansion and is perhaps a primary cause of ascites.

It is usually thought that initial ascites formation does not depend on hormonal and renal activity; rather, these try to adjust the body to the novel distribution of water, protein, and electrolytes. Sherlock[1] places most emphasis on portal hypertension as the initiating and localizing force in ascites in portal cirrhosis. Hypoalbuminemia prompts the flow at lower portal tensions, increased hepatic lymph enhances formation and localization, and the secondary relative hyperaldosteronism unfortunately perpetuates and worsens the condition.

ASCITIC FLUID

The ascitic fluid which accumulates in cirrhosis is usually clear, green, or straw-colored.[1] It has a specific gravity of less than 1.016, protein content is less than 3 gm %, electrolyte values are similar to those of blood, and cells in a centrifuged sample are less than 300/cu. mm., being mainly mesothelial cells and histiocytes.[14] In the abdomen, the fluid and protein of ascites constantly exchange with blood in the peritoneal capillaries and lymph in the lymphatics. Forty to 80% of water content exchanges every hour.[1] The pressure developed in the abdomen rarely excedes 10 mm Hg greater than that in the right atrium; anything more is so uncomfortable it must be relieved by a small paracentesis.

ASCITES IN OTHER DISEASES

Ascites may develop in other diseases of the liver.[1] Biliary cirrhosis produced by a chronic cholestasis distorts intrahepatic vasculature and in three to five years damages liver cells sufficiently to precipitate ascites. About one-half of patients with malignant hepatomas have ascites which may be due to the accompanying cirrhosis, to portal or hepatic vein invasion causing thrombosis and hypertension, or to peritoneal involvement producing a hemorrhagic exudate. Extensive liver metastases may have the same effects. Severe hepatitis may also produce ascites.

Two other groups of diseases causing significant ascites are chronic right heart failure and diseases of the peritoneum. The etiology of the first may be valvular, chronic myocarditis, adherent pericarditis, or chronic lung disease with pulmonary hypertension and right heart dilatation or hypertrophy.[1] Decreased right ventricular emptying results in congestion in the hepatic veins and pericentral sinusoids, with centrilobular parenchymal necrosis and hemorrhage. Increased deposition of reticulin and collagen occurs around the hepatic vein walls, and eventually, fibrous tissue bands connect the hepatic veins. Nodular regeneration is not conspicuous. Ascites develops in advanced congestive failure, the product of portal venous hypertension and hypoalbuminemia created by the hepatic outflow block and hepatocellular damage. The low output aspect of heart failure is an obvious but not clearly proven mechanism of secondary hyperaldosteronism. The progression from chronic hepatic congestion to frank cardiac cirrhosis

is rare and requires either repeated episodes of failure or continuous failure for over sixty days. It is seen most acutely in patients with tricuspid insufficiency or cor pulmonale of any cause. At this stage, the ascites becomes intractable.

Portal cirrhosis is not infrequently associated with one form of congestive heart failure[1]. In a comparison, the mean heart weight by decades was found to be higher in patients with portal cirrhosis than in the normal population; and 48% of a series of 108 patients with Laennec's cirrhosis were shown to have serious heart disease. These patients develop a hyperdynamic or hyperkinetic state, pumping an increased blood volume through a dilated peripheral system, and large numbers of arterio-venous shunts. Arterial oxygen unsaturation makes further demands on the heart, and the myocardium hypertrophies and dilates. As well, alcohol may have a toxic effect on heart muscle resulting in cell death and fibrosis. Congestive failure may ensue with its signs superimposed on those of cirrhosis.

Peritoneal disease causing ascites is usually due to metastatic implantation, occasionally to inflammation.[15] An implant of cells irritates the peritoneum stimulating increased vascularity and permeability and exudation of serum and cells into the abdominal cavity. The source of the implant is commonly stomach or ovary carcinoma; the fluid produced has a high protein content, greater than 500 cells/cu. mm., and is often bloody. In two cases of ascites caused by myeloid tissue implantation from spleen, peritoneal fluid revealed a distinct cytological pattern – nucleated red blood cells, immature granulocytes, and occasional megakaryocytes[16] In most cases, however, malignant cells are not unequivocably demonstrated.

Inflammation of the peritoneum may be aseptic for example, peritonitis caused by pancreatic enzymes liberated in acute pancreatitis.[17] It may also be septic, as in tuberculous peritonitis. Both begin as a fibrinous exudate from inflamed tissue followed by an inflammatory cell infiltration giving purulent ascites with a high protein content. Blood vessels and lymphatics may be damaged resulting in hemorrhagic and chylous ascites. Tubercle bacilli are seldom cultured from tuberculous ascites.

SUMMARY

The symptoms and signs of ascites are presented. Its pathogenesis is described in detail using ascites of portal cirrhosis as an example. It is suggested that portal venous hypertension, increased hepatic lymph production, and hypoalbuminemia are primary causes of ascites and that a secondary hyperaldosteronism develops, perpetuating and worsening the condition. Finally, ascites associated with chronic right heart failure and diseases of the peritoneum is discussed briefly.

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(Continued from page 142)

detection of preformed antibodies. Of the twenty-one transplants for which histocompatibility tests were done since May, 1967, only three were judged to be completely compatible.[12]

SUMMARY

Despite the immense problems remaining, the prospects for prolonged graft survival are encouraging. This is due to improved surgical technique, better methods of liver preservation in transit, better quality of donated livers, improved tissue matching, improved immunosuppression and increased knowledge of postoperative complications.

David W. Page 7T0

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Clinicopathological Conference

Cirrhosis, Ascites And Nephritis In An Adolescent Female

CASE PRESENTATION

Miss J. K., a 14-year-old girl, was transferred to TGH following extensive investigation at the Kitchener-Waterloo Hospital.

She had been well until ten weeks prior to hospital admission. From this time she was troubled by poorly localized abdominal discomfort after meals. The patient also noticed that her "skirts were tight". She suffered a severe bitemporal headache on 3 successive days.

Apart from these complaints, the patient remained relatively well until 6 weeks later, when she suffered a bout of severe right lower quadrant pain associated with chills and fever which lasted for 4 hours. When seen by her doctor there were no physical signs, and the urine was clear.

Over the ensuing 2 weeks, bilateral swelling of the ankles developed, arousing the concern of her mother. With progressive abdominal distention as well, the patient was admitted to hospital in Kitchener. A provisional diagnosis of "acute glomerulonephritis" was made.

On functional enquiry, the patient described frequent mild upper respiratory infections and recurrent nosebleeds for 2 years. However, there had been no colds or sore throats for 9 months. She had occasional aches and pains over the joints of her hands and fingers and occasional myalgic pains in her calves. At no time, however, had there been signs of joint inflammation. Menses had commenced at age 12, and had occured irregularly at about 6-week intervals until their cessation at the time of onset of symptoms. There was no history of skin rash, jaundice, or symptoms referable to the cardiovascular respiratory or urinary systems. The patient denied fever, photosensitivity and Raynaud's phenomenon.

The patient's mother, of Swiss descent, displayed a sallow complexion similar to that of the patient. The family history was otherwise non-contributory. Neontal and birth history were unremarkable.

Physical examination revealed an alert, pleasant young lady. Apart from her sallow complexion she did not appear chronically ill. There was no scleral icterus.

There were no abnormalities of the head, neck or chest. The Fundi were normal; there was no lymphadenopathy.

The blood pressure was 150/85 and the apex beat 80 per minute. There was no clinical cardiomegaly and JVP was not increased. A grade I/IV systolic murmur was heard at the left sternal border. Ankle edema was not significant at this examination.

The abdomen was grossly distended with a fluid thrill and shifting dullness easily elicited. Superficial veins were noted to be prominent, although they did not form a definite caput medusa. The positions of liver and spleen were determined by ballottement: the liver extended just below the right costal margin; the spleen 5 cm below the left costal margin. Genitalia and pubic hair distribution were normal and rectal examination was unremarkable.

Striae were present on the upper thighs and abdomen. As well, numerous *café-au-lait* spots were observed on the trunk and right arm. At this time, there were no petechiae of the skin or mucous membranes. Spider telangectasia and palmar erythema were absent.

Neurological and musculo-skeletal examination were within normal limits.

Initial hematological studies showed pancytopenia. Hb was 9.8; WBC 2,800, with slight eosinophilia; platelets 52,100; retic. count 1.8%. The smear showed some macrocytosis with moderate poikilocytosis. A red cell fragility test was normal. An LE test was negative.

The urine showed changes commensurate with glomerulitis. Gross proteinuria was present. On microscopic examination many RBC's, WBC's and casts (hyaline and granular) were found.

Blood chemistry revealed evidence of liver involvement: CCF was 3+, SGOT 498, Alkaline phosphatase 30. Prothrombin time was 18 seconds. Total serum protein was 5 gm%, with an albumin of 1.7 gm%. Serum electrolytes were normal and the BUN was 13.

Radiologic studies showed some prominence of hilar shadows in the chest. On intravenous pyelography, there was good bilateral function of the kidneys.

DIFFERENTIAL DIAGNOSIS

The positive features of the disease included anemia, thrombocytopenia and leukopenia probably on the basis of hypersplenism. There was impairment of liver function with elevated transaminase, ascites and peripheral edema. Nephritis was a prominent clinical feature. Additional features were *café-au-lait* pigmentation and secondary amenorrhea.

Clinical conditions considered at this time in the differential diagnosis were:

1) Multi-system collagen disease (a) systemic lupus erythematosus; (b) polyarteritis nodosa: This must be considered in many difficult diagnostic problems. Liver involvement however, is rare and only mild splenic enlargement occurs.

2) Subacute glomerulonephritis could cause the urinary changes, edema and anemia seen in this case; but hepatitis and splenomegaly are difficult to explain on this basis.

3) In Gaucher's disease, hepatosplenomegaly is prominent without lymphadenopathy. Involvement of the kidneys may occur. Spots of yellowish-brown pigmentation may be seen in the skin. In this condition, there frequently are erosions of the marrow producing roentgen findings. Typical Gaucher cells filled with the characteristic cerebroside are seen in the marrow.

4) Neoplastic disease involving the liver and kidney: Hodgkin's disease and malignant lymphoma were considered as possible etiologies.

5) Banti's syndrome (chronic congestive splenomegaly) is a disease of young adults beginning insidiously with vague abdominal complaints. Splenomegaly and anemia are the chief clinical findings, with cirrhosis frequently occurring as the disease progresses. In 60% of cases a cause can be found (eg. cirrhosis, cavernous transformation or thrombosis of the portal or splenic veins). Renal disease is not a feature.

6) Cirrhosis of the liver: Wilson's disease presents within the first 3 decades of life. Abnormalities of renal tubular reabsorption are found including proteinuria, jaundice, splenomegaly, ascites: pancytopenia may be a prominent feature, but in most cases the neurological signs and symptoms are most prominent, both at presentation and during the subsequent course of the disease. Primary biliary cirrhosis may occur in young women but pruritis, a prolonged course and chemical indices of pronounced obstruction are typical. Cirrhosis in chronic active hepatitis is discussed later.

7) Neurofibromatosis: is an inherited disorder of increased skin pigmentation and multiple neuro-fibromas. Numerous *café-au-lait* spots (as in this case) are pathognomonic. In 5-10% of cases, sarcomatous change may occur in the tumours. However, these are characteristically found in the subcutaneous tissue or CNS.

8) Venous obstructive disease, of the inferior vena cava, hepatic vein or portal vein, by tumour or thrombosis.

9) Amyloidosis may cause proteinuria nephrosis and hepatomegaly, although impairment of liver function is unusual.

FURTHER INVESTIGATIONS, COURSE AND TREATMENT

Protein electrophoresis revealed a gamma-globulin of 2.1 gm% (upper limit 1.6) with a narrow spike zone "more in keeping with a dysproteinemia than a connective tissue disorder." The urine was negative for Bence-Jones protein. The ASO titre was not elevated. A Congo red test revealed a normal dye retention of 86%.

A bone marrow study was within normal limits, including a negative LE preparation. There were no Gaucher cells.

An inferior vena cavagram revealed a 50% narrowing of the intrahepatic portion; however, normal pressures were recorded in this segment and no significant obstruction was felt to be present. A liver scan with radioactive gold revealed normal reticuloendothelial function with the liver slightly enlarged and the spleen measuring 16 x 7 cms. Slit lamp examination failed to reveal Kayser-Fleischer rings in the cornea.

Apart from her ascites the patient remained relatively comfortable while in hospital. She was treated with prednisone 15 mg q.4.h. and folic acid 5 mg daily. After 3 weeks of hospitalization in Kitchener, she was sent to TGH for further investigation and management.

At this time she exhibited a chronically ill appearance with slight icterus. Gross, pitting edema was observed over the back, the vulva and the legs with the diameter of the left leg 1″ greater than the right. Bruising was noted on the upper arms as were petechiae over the thighs. Examination of the chest revealed a right pleural effusion which was confirmed by x-ray.

The patient's WBC was now 15,000 (94% polymorphs). There was intermittent pyrexia, but no focus of infection could be found. The platelet count had fallen to 40,000. The direct Coomb's test was positive. ESR was 60. The LE phenomenon could not be demonstrated on 4 repeated attempts.

Liver function tests were as follows: van den Bergh 3.1 mg%. Urine negative for bile and urobilin. SGOT 55; alkaline phosphatase 54; prothrombin time 18 seconds; BSP retention 54%.

A 24-hour urine protein was 0.3 gm. This was interpreted as an improvement resulting from steroid therapy.

IVP now showed obstruction of the right ureter at the pelvic brim with hydroureter and hydronephrosis. Both IVP and barium enema were suggestive of a pelvic mass. A pelvic examination revealed suprapubic dullness and the impression of a mass in the right lower quadrant.

Initial treatment consisted of Lasix 80 mg b.i.d. and Aldactone. There was a 12-lb. diuresis. Ascites reformed subsequently and a paracentesis 12 days after admission yielded 3,000 cc of straw-coloured fluid. No pelvic mass could be palpated following the procedure.

By this time, the patient's condition was deteriorating with persistence of severe anasarca and progressive weakness and drowsiness.

The BUN rose to 40 mg%. Prothrombin time was 28 seconds. The gamma-globulin had risen to 3.6 gm% and the albumin fallen to 1.5 gm%. Electrolytes revealed that Na+ had fallen to 120 meqs./l.

Three days after paracentesis the patient was in a stuporous condition. She had a convulsion of 3 minutes duration and died.

AUTOPSY FINDINGS

The right pleural cavity contained 20 ml. of amber fluid. The gross and microscopic appearance of the lungs were unremarkable.

The heart was grossly normal with no lesions of valves or of coronary arteries. The pericardium showed no evidence of inflammation or vasculitis. The inferior vena cava was occluded by a recent thrombus, but no evidence of chronic obstruction of the major veins was apparent.

The abdominal skin displayed striae with marked edema of the epidermis and inflammatory cells in dermis and epidermis. The abdominal cavity contained 2500 cc of ascitic fluid. Surprisingly no mass lesions were found in the abdomen or pelvis. Examination of the gastro-intestinal tract revealed no gross or microscopic abnormality.

The liver weighed 660 gm and had an irregular nodular surface. On section, chronic inflammatory cells were seen; thick bands of fibrous tissue separated irregular nodules averaging 0.5 cm in diameter. The latter changes were similar to those seen in posthepatitic cirrhosis.

The spleen was grossly enlarged with two large areas of recent infarction. Changes of congestive splenomegaly were apparent. Under high power, hematoxyphil bodies were seen. There were, however, no "onion-skin" lesions (periarteriolar fibrosis) of the splenic arterioles.

The kidneys displayed thickened cortices. The right ureter was surrounded by fibrous tissue at the level of the pelvic brim and dilatation of the right ureter and calyceal system was present. Microscopic section revealed swollen glomeruli with thickening of the mesangial tissue and of the basement membranes. There were focal fibrinoid deposits in the glomeruli, and a rare hematoxyphil body was seen.

DISCUSSION

On the basis of autopsy findings, this case might be designated as systemic lupus erythematosus (SLE). There were renal changes of glomerular swelling, basement-membrane thickening and focal fibrinoid necrosis, although the typical "wireloop" lesion was not seen. Hematoxyphil bodies (which, in conjunction with periartetriolar fibrosis in the spleen, constitute the most characteristic microscopic findings) were present. Other pathological manifestations, e.g. vasculitis, were absent, possibly as a result of corticosteroid therapy.

Nonetheless, consideration of the severe liver disease present in this case causes some difficulty. One must consider the possibility that the clinical syndrome was the result of a disease process occurring principally in the liver, i.e., "lupoid hepatitis" or chronic active hepatitis."

CHRONIC ACTIVE HEPATITIS

Since 1950 clinical investigators have recognized a severe progressive form of hepatitis, described originally in young women[1]. This disease entity was characterized by marked infiltration of the liver by plasma cells and lymphocytes, and by a progressive course leading to cirrhosis, and death after several years. A feature which attracted much interest was the frequent occurrence of the clinical features associated with SLE, and the presence, in a significant majority, of a positive LE test.

Attention was focused on various facets of this disease which came to be known by a multitude of confusing names[2]. These included "active chronic hepatitis", "lupoid hepatitis", "plasma cell hepatitis" and "cirrrhosis of young women." Most of these names embodied incomplete knowledge of the clinical features, and a presumption of the etiology. As the etiology is, in fact, still unknown, it seems best to use the non-specific term "chronic active hepatitis".

This clinical syndrome is not rare, and moreover, its incidence may be increasing. Sherlock[3] described a series of 140 cases which she had investigated over a 6-year period. It is not in fact, a cirrhosis of "young women", as 20% of the cases are described in males and half of the cases are over 50 years of age when the disease is first recognized. Chronic active hepatitis may account for many patients who were previously termed "cryptogenic cirrhosis".

The disease usually has a gradual onset with persistent jaundice as the first sign of liver impairment. The onset is incompatible with viral hepatitis both clinically and epidemiologically[4]. There is no relationship with alcohol excess or biliary tract disease, and this represents one of the few cases of persistent hyperbilirubinemia where this is true.

There may be fever, fatigue and arthralgia at the onset of the disease. Secondary amenorrhea is frequent, especially in younger females and was a feature of the above case. Typical physical findings, e.g., striae and acne, mentioned in early descriptions are in fact inconsistent features. Generally the patient experiences relatively little discomfort until terminally. However, the course may be punctuated by multi-system disease with signs and symptoms similar to those of a variety of immune diseases. These include ulcerative colitis, Hashimoto's thyroiditis, scleroderma, rheumatoid arthritis or Coombs-positive hemolytic anemia, the latter being a feature of the case under discussion. There may in fact be features typical of SLE, including skin rash and pericarditis.

Laboratory tests serve to distinguish chronic active hepatitis from other forms of liver disease. There is persistent hyperbilirubinemia. The transaminases are persistently elevated, providing evidence of "activity" of the disease. The SGOT is often strikingly greater than the SGPT (the reverse is typical of viral hepatitis). The serum albumin is usually well preserved until the late stage. There is a marked elevation of gamma-globulin, frequently to higher levels than that seen in SLE. This feature is considered a manifestation of disease "chronicity". "β-δ bridging" seen on the protein electrophoretic strip is frequently found, but is absent in SLE. The sedimentation rate is significantly elevated. The LE test is positive in only 15% of cases; although the present case does not fit this pattern, the test tends to be positive in patients exhibiting arthritis and a more fulminant course. The prothrombin time is often disproportionately elevated, and frequently interferes with the assessment of patients by liver biopsy.

Biopsy and autopsy studies divide the pathological changes into two phases[5]. In the earlier hepatic stage, there is patchy liver cell necrosis with Kuppfer cell hyperplasia and infiltration by mononuclear cells. In the second stage there is aggressive fibrosis with the development of macronodular cirrhosis, indistinguishable from postnecrotic cirrhosis.

Although there may be some minor remissions, the overall trend of the disease is progressive, with death usually occurring in 6 months to 10 years. Controlled studies suggest that early institution of steroid therapy in the hepatitic stage may delay progression[6]. However, this mode of therapy is much less effective in this disease than in cases of progressive liver dysfunction following viral hepatitis. Immunosuppressive agents may offer some advantage, although clinical trials are incomplete.

The etiology of this disease is unknown although current thought stresses immunological derangements. Hypergammaglobulinemia, LE cells, and the association with other possibly "auto-immune" diseases are certainly in keeping with this theory. Circulating antibodies to certain liver components have been demonstrated although they are frequently found in biliary cirrhosis and infectious hepatitis as well. A variety of experimental models has failed to demonstrate a cytotoxic effect of these antibodies.

The case of Miss J. K. demonstrates many of the features described in chronic active hepatitis. However the rapid course and severe renal involvement are not typical. In this respect, the relationship of chronic active hepatitis to SLE is of great interest.

Most reported cases of chronic active hepatitis have displayed little or no renal involvement. Vasculitis periarteriolar fibrosis and other microscopic changes of SLE have been minimal or absent. In contrast, classical SLE displays little involvement of the liver. A recent review of 375 cases[2] revealed no deaths due to liver failure and no cases of cirrhosis. Microscopic change in the liver was infrequent and minor.

A difficulty therefore arises, in the rare patients presenting "lupoid" involvement of both liver and kidney. Several authors[2] have described patients exhibiting the history and findings of chronic active hepatitis with moderate to severe renal lesions similar to SLE. They felt that the presence of cirrhosis was the determining factor in classification and referred to these patients as chronic active hepatitis with nephritis. It would appear that the patient under discussion belongs to this group.

The difficulties in consideration of these two diseases might be overcome by postulating a spectrum of manifestations. There is, however, recent immunological evidence which militates against such a proposal. Whittingham, et al[7], analyzed a large series of sera for antibodies to smooth muscle. This factor was found in 60% of cases of chronic active hepatitis, but was present in less than 5% of patients with other liver diseases, and absent from the sera of patients with SLE and other collagen diseases.

The discovery of smooth muscle antibodies is an important breakthrough in diagnosis. On the other hand, cytotoxic properties of these antibodies have not been substantiated. The etiology of chronic active hepatitis, like that of the other so-called "auto-immune diseases,,' still awaits further elucidation.

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Student Research

Neonatal Hyperbilirubinemia and Hepatic Detoxification In The Fetus And Neonate

INTRODUCTION

Metabolic detoxification is one of the most important hepatic functions. Drug metabolizing enzymes that perform this function are largely microsomal and include hydroxylases, oxidases, demethylases, hydrolases, conjugases and several others.

The glucuronylating system is an important means of disposal for both drugs and natural metabolic products. It consists of two distinct steps outlined in Figure 1.

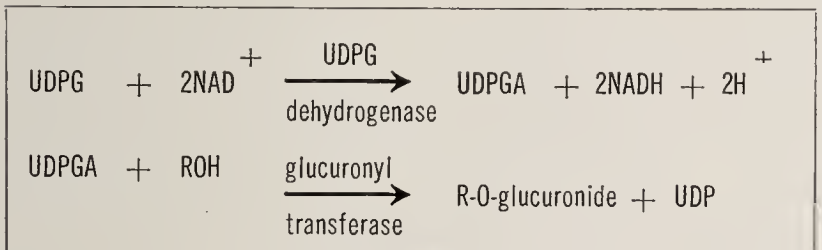

Fig. 1. The glucuronylation reactions

The first reaction involving the formation of the donor compound uridyl diphosphate glucuronic acid (UDPGA) is catalized by the extra-microsomal supernatant enzyme UDPG dehydrogenase. The second is catalized by a microsomal transferase. Bilirubin is one of the many compounds metabolized in this manner. Due to its low water solubility, it cannot be excreted in the bile or by the kidney, while the water-soluble glucuronide is readily excreted.

The fetus and neonate of certain species have been found to show evidence of impaired detoxifying capabilities. Recently Bernstein and co-workers[1], studying bilirubin metabolism in the fetus with new intrauterine operative techniques, showed that in the monkey, with the placenta acting as a major physical detoxifying route, the fetal liver possesses only a fraction of the detoxifying activity of the adult liver. In contrast, the liver of the dog fetus was found to be comparable to that of the adult in its handling of the detoxifying function. These findings seem to correlate well with the lack of evidence of physiologic jaundice in the newborn dog *vis a vis* its frequent occurrence in the newborn monkey.

Most infants develop transient hyperbilirubinemia (over 1 mg. % unconjugated bilirubin) during the first week of life and 20-60% of them suffer from neonatal icterus (over 10 mg. per 100 ml), the frequency depending heavily on the birth weight.[2] This condition has been reported to be related to poor performance in subsequent tests of psychomotor development. However, more recent reports seem to indicate that brain damage occurs only in areas where the reduced oxygen utilization caused by the high levels of bilirubin in the blood is superimposed on the effects of hypoxic damage previously incurred, possibly at birth.[3]

Regardless of the exact mechanism of its pathogenicity, neonatal icterus is considered dangerous.

BASIS OF EXPERIMENTAL DESIGN

The biochemical defect responsible for non hemolytic physiological neonatal hyperbilirubinemia is thought to be the low activity of the enzyme glucuronyl transferase but this is not yet certain. Recent work with premature babies using needle biopsies from the liver has failed to correlate the fall to normal levels of bilirubin by day fifteen of life on the one hand with an unchanged glucuronylating activity on the other.[4] An alternative recently proposed is based on studies implicating an intracellular organic anion-binding protein concerned mainly with hepatic uptake of bilirubin.[5]

There is no doubt that the glucuronylating activity in the perinatal animal of some species, including primates, is low. The biochemical apparatus is either underdeveloped or suppressed. The first possibility is teleologically reasonable since the placenta seems to play a major role in detoxification during intrauterine life[1] but evidence as to the second possibility is also available and will be outlined later.

One line of investigation which might throw more light on this problem of whether the biochemical apparatus is either underdeveloped or suppressed involves the property of the drug metabolizing enzymes to be induced *in vivo* by a wide variety of polycyclic and halogenated hydrocarbons, barbiturates and other compounds. Induction is most likely due to enhanced *de novo* synthesis; it does not occur *in vitro* and is blocked by inhibitors of protein synthesis.

The phenomenon of induction has been well documented, and is considered to be of major importance in both chronic drug therapy, where it can account for the development of tolerance, as well as in the concomitant administration of two or more drugs, one of which might enhance the metabolism of the others.

Therapeutic enhancement of bilirubin metabolism in the newborn by the administration of the inducer phenobarbital is gaining increasing acceptance. After it was shown to be effective in the treatment of patients with congenital familial hyperbilirubinemia[6,7] other studies

demonstrated its value in the treatment of neonatal icterus.[2 8] This effectiveness was observed in hyperbilirubinemia occuring in a variety of settings such as ABO incompatibility, glucose - 6 - phosphate dehydrogenase deficiency, cephalhematoma and others. Administration of inducer to the pregnant mother yielded more favourable results than administration to the newborn. However, similar treatment of pregnant animals showed little effect on the fetal hepatic drug metabolising enzyme activity.

METHOD AND RESULTS

These studies were undertaken in the hope of further elucidating the mechanism of the above described phenomena. The detoxifying capability of whole animals were determined by the *in vivo* "sleeping test". Hepatic drug-metabolizing enzyme activity was determined by *in vitro* assay. Determinations were made on pregnant and non-pregnant rats.[9]

The "sleeping test" consisted of injecting pentobarbital, 15 mg/kg. intraperitoneally and noting the length of time required before the animals exhibited the first sign of movement and before they completely awoke as indicated by an immediate search for food. A significant difference between the pregnant and non-pregnant animals was found with the pregnant ones sleeping longer (Table 1). Longer sleeping times are of course interpreted as more sluggish detoxification.

TABLE I: Effect of Pregnancy as Demonstrated by the Sleeping Test

	Pentobarbital dose, mg/kg.	Non-pregnant rats	Pregnant rats	Significance of difference
First sign	15	111.2 ± 11.7 (5)	137.0 ± 19.1 (5)	$p < 0.02$
Comple awakeness	30	147.5 ± 1.4 (5)	170.6 ± 8.9 (1)	$p < 0.01$
Complete	15	138.2 ± 12.7 (5)	195.7 ± 9.6 (5)	$p < 0.01$
awakeness	30	192.5 ± 1.5 (5)	256.4 ± 10.8 (7)	$p < 0.001$

All means and standard errors are in minutes. The number of animals is in brackets.

The activities of two representative drug-metabolizing enzymes were determined *in vitro: o*-aminophenol glucuronyl transferase and 4-methylcoumarin hydroxylase. The transferase was assayed by incubating o-aminophenol and ascorbic acid with liver microsomal fraction and UDPGA. The hydroxylase was assayed by incubating 4-methylcoumarin with liver microsomal fraction and an NADH/NADPH regenerating system. The amounts of substrate transformed were determined in each case by spectrophotometric methods. The enzyme activity in the liver of non-pregnant rats was found to be significantly higher than in the liver of pregnant rats.

Induction of the drug metabolizing enzyme activity in the liver was achieved by the administration of phenobarbital, 4-methylcoumarin and 3-methylcholanthrene to the rats. Enzyme activities were determined as described above. They were found to be inducible in the liver of pregnant rats to levels 3-4 times as high as in the untreated animals and equal to or even exceeding those in non-pregnant rats (Table II).

TABLE II: The effects of **in vivo** treatment with phenobarbital or 4-methylcoumarin on the o-aminophenol transglucuronylase and 4-methylcoumarin-3-hydroxylase activities in the liver of non-pregnant and pregnant rats.

Enzyme		No Treatment	Phenobarbital	4-Methylcoumarin
o-aminophenol transglucuronylase	NON-PREGNANT	0.180 ± 0.032 (8)	0.261 ± 0.029 (5)	0.471 ± 0.052 (4)
	PREGNANT	0.066 ± 0.008 (8)	0.109 ± 0.008 (5)	0.178 ± 0.024 (4)
4-methylcoumarin	NON-PREGNANT	1.70 ± 0.32 (15)	3.32 ± 0.31 (4)	4.00 ± 0.17 (8)
	PREGNANT	0.82 ± 0.15 (18)	2.43 ± 0.24 (4)	3.39 ± 0.19 (7)

Enzyme activities are in nmoles substrate transformed hr/mg protein. The number of animals is in brackets.

This increase demonstrates that a reserve capacity for drug metabolizing enzyme synthesis persists during pregnancy. The ability to be induced relative to levels of enzyme activity in the non-induced state does not seem to be decreased during pregnancy.

DISCUSSION

Arias and Gartner[11] demonstrated that unconjugated hyperbilirubinemia could be produced in human babies by feeding pregnanediol in amounts equivalent to that in human milk. Milk from the mothers of jaundiced infants was found to inhibit glucuronyl transferase activity *in vitro*. A marked increase in hepatic drug metabolizing capacity has been noted in mice around the age of 3 weeks, a period coinciding with weaning from the mother.[12] Early weaning has been shown to lead to an acceleration of hepatic drug metabolism in the newborn rat.[9] The rat is the only animal, in which sex differences

in drug metabolizing activity have been observed. In particular, the male was found to exhibit more activity than the female with its activity being reduced upon treatment with estradiol.[13]

The experimental results presented in this article and the above evidence seem to point to an inhibitory maternal influence possibly of hormonal origin, being at least partly responsible for the low drug metabolizing capabilities of the fetal liver. The suggestion has been put forward[9] that the factor involved is a female gonadal steroid hormone. The levels of such hormones rise very markedly during pregnancy and fall rapidly prior to parturition. In the rat, the hepatic microsomal enzyme activity seems to rise slightly just prior to birth, then rises rapidly to a peak at about 3-5 days post-partum only to fall back and become stable at levels comparable to those in the adult after about three weeks.

Administration of inducer to pregnant rats produced a much smaller (50%) rise in drug metabolizing activity in the fetus than in the mother. The reason for the lower capacity of induction by the fetal liver enzymes is not clear. Since the inducer drugs cross the placenta quite readily, the placenta cannot be implicated. Many other factors could be involved, including the availability of co-enzymes and the levels of inducer and perhaps of inhibitors at their sub-cellular sites of action.

Further elucidation of the mechanisms involved is needed. Such studies will expand our concepts of fetal and neonatal pharmacology and result in improved neonatal therapy.

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Recent Advances — *Liver Transplantation - A Brief Look*

INTRODUCTION

In 1963, fifteen thousand people between the ages of five and sixty died of liver disease in the United States, ninety per cent of who were alcoholic cirrhotics. Figures from England and Wales approximated the incidence per capita observed in the U.S.A.[1]

Liver transplantation potentially offers a method of altering the barren prognosis for patients with irreversible hepatic damage. Diseases of the liver for which liver transplantation has been employed to date include congenital biliary atresia, hepatoma, post-necrotic cirrhosis and alcoholic cirrhosis. Others which might be amenable to transplantation are primary biliary cirrhosis, hepatolenticular degeneration, irreparable biliary duct injury, hemochromatosis, and liver-based inborn errors of metabolism.[2]

HISTORY

The short history of liver transplantation began with Welch's description of whole organ auxiliary liver transplantation in dogs in 1955. The first efforts at experimental orthotopic transplantation originated with Cannon in 1956. Dr. Francis D. Moore established a well-organized program of orthotopic liver transplantation at the Peter Bent Brigam Hospital in Boston in June, 1958. Later that summer, Starzl began his experiments in Chicago.[3]

In Denver in 1963, Starzl et al attempted five orthotopic homotransplantations in humans, all of whom died within twenty-two days of the procedure. Similar reports were made by Moore, et al, in Boston and Demirleau, et al, in Paris in 1964. In October, 1967, Starzl reported four additional transplantation survivors, the longest survivor achieving ninety-four days with a new liver. By 1968 Calne reported one patient who survived one hundred and thirty-two days. In his recent book Starzl describes a patient who survived for three hundred and eighty days before a second transplantation was performed, as well as one patient who was operated on

in February, 1968 and whom he reported as still alive on April 15, 1969.

PRESERVATION OF DONOR LIVER

Under normothermic conditions, severe ischemia of but short duration is permissible with expectation of preservation of function. Starzl reports irreversible brain damage used as the criterion for death in some of his donors, while others suffered cardiac standstill.

Because external cardiac massage presents the danger of liver laceration, an infusion method is preferred using a balanced electrolyte solution cooled to 2°C. Donor hepatectomy completed, the method of ex vivo hepatic perfusion described by Brettschneider et al[4] is employed, using a combination of hypothermia, hyperbaric oxygenation, and perfusion with dilute blood.

THE TECHNIQUE OF TRANSPLANTATION IN BRIEF[5]

After the surgeon opens the abdominal cavity, the *porta hepatis* is identified, dissected out and the feasibility of transplantation determined according to criteria established for the various hepatobiliary diseases. With the falciform ligament incised to the suprahepatic vena cava, the right and left coronary and triangular ligaments are likewise divided. All vessels entering and leaving the liver are skeletonized and occluded and divided and the faulty organ removed.

Although exacting in technical skill, the placement of the new organ into the recipient body involves simply (1) the reestablishment of the major vascular channels, establishing bile drainage (usually via cholecystoduodenostomy), and suturing the falciform ligaments conjointly for fixation.

This outline of Starzl's technique is merely that. Major problems such as size discrepancy, operative staging, technical modifications with vascular anomalies, etc. are obviously beyond the scope of this discussion.

POSTOPERATIVE COMPLICATIONS

Hemorrhage postoperatively, resulted either from local oozing or from a general hemorrhagic diathesis. Blecher et al working with pigs found an immediate postoperative increase in fibrinolytic activity. Hutchinson's results were similar.[6] Hutchinson also described platelet sequestration in the Space of Disse minutes following graft revascularization in dogs. Starzl described increased fibrinolysins, hypofibrinogenemia, thrombocytopenia, and the appearance of heparin-like substances in dogs.[7] Later, von Kaulla[8] speculated that intravascular coagulation coupled with decreased production of proteins resulted in the bleeding diathesis. This view is supported by Starzl who stated, "The finding that all measured clotting factors declined far more rapidly than would have been expected from their biological half-lives, and that these alterations were accompanied by thrombocytopenia and fibrinolysis were characteristic of a consumptive coagulopathy.[9]

In his recent book, Starzl reports the occurrence of the following complications in addition: total arterial thrombosis, lobar arterial thrombosis, portal vein thrombosis, air embolism, adrenal infarction, right diaphragmatic paralysis, hepatic abscess, septicaemia, and intestinal injury.[10] More acute problems include gastrointestinal ulceration or bleeding, pulmonary embolization, pancreatitis, and arterial hypertention.

GRAFT REJECTION

As hepatocytes contain erythrocyte antigens,[11] red blood cell comptability is required. Rejection comparable to the cell-mediated type hypersensitivity was observed in all orthotopic liver transplants which lived beyond one month in the series of twenty-five transplants performed by Starzl between June, 1967 and February, 1968. Other centres reported similar results. Clinically, these rejection events took several forms, including subclinical rejection, crisis rejection within six to twenty-nine days, and indolent rejection beginning within six days and progressing insidiously for four to seven weeks during which time the patients did not appear severely ill.

Ultimately, those patients with indolent rejection required retransplantation, while treatment for the first two groups were managed with modifications in their immunosuppressive regimen.

PREVENTION OF REJECTION

The three agents most successful in suppressing graft rejection are azathioprine, heterologous antilymphocyte globulin (ALG) and prednisone. Total body irradiation met with complete failure in reported experiments with dogs.

Starzl describes two regimens: one, utilizing double drug therapy of azathioprine and prednisone as well as less important ancillary drugs (azaserine and Actinomycin C). There were no survivors beyond thirty days on this regimen. Triple drug therapy included the two drugs mentioned and ALG. To both of these regimens may be added local homograft irradiation. Although the way in which the three drugs are used together varies with each case, triple drug therapy is now the accepted method of treatment.

Insuring histocompatibility is a further method of prolonging transplant survival. Terasaki describes the logistics of obtaining antigen profiles. For example, in one analysis twelve histocompatibility groups were taken into consideration, thirty-eight prospective recipients of blood type O recipients, creating four hundred and ninety - four possiblee donor-recipient combinations to be considered. Other problems include elimination of false values (both negative and positive), reproducibility, and

(Continued on page 134)

Grand Round Quiz

Liver

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1. *The bromsulphalein excretion test (BSP) is a sensitive index of which of the following phases of hepatic function?*
   A. hepatic circulation
   B. hepatocellular function
   C. hepatic excretion
   D. all
   E. none

2. *Which of the following is the most important factor in the production of ascites?*
   A. proximal renal tubular sodium and water retention
   B. variations in the peritoneal membrane
   C. defective albumin synthesis
   D. secondary aldosteronism
   E. increased hepatic lymph

3. *Anemia associated with chronic liver disease may be due to the following:*
   A. hypersplenism
   B. nutritional deficiency
   C. hemolysis
   D. bone marrow depression
   E. all of the above

4. *The earliest clinical sign in ascites is:*
   A. ballottment of the liver and spleen
   B. resonance to percussion in the flanks
   C. distension of abdominal wall veins
   D. dullness to percussion in the flanks
   E. a sudden weight gain

5. *The most common cause of jaundice between the ages of 40 and 60 years is:*
   A. choledocholithiasis
   B. pre-hepatic disorders
   C. hepatitis
   D. cirrhosis
   E. none of the above

6. *The jaundice produced by the anaesthetic agent halothane is due to:*
   A. cholestasis
   B. hepatitis - like reaction
   C. competition with conjugated bilirubin for excretion
   D. hemolytic reaction
   E. interference with bilirubin conjugation.

7. *Which of the following is not considered part of the treatment of acute hepatic coma or pre-coma due to hepatitis:*
   A. neomycin by mouth
   B. exchange blood transfusion
   C. high caloric, zero protein diet
   D. administration of morphine derivatives for sedation
   E. empty bowels of nitrogen-containing materials

8. *A 57-year-old cirrhotic presents to the emergency room with copious haematemesis. The most important aspect of initial management is:*
   A. intravenous therapy
   B. intubation to prevent aspiration of blood
   C. introduction of the Sengstaken-Blakemore tube
   D. emergency esophagoscopy
   E. measurement of splenic pulp pressure

9. *A 23-year-old male presents with a 2-week history of sore throat, feverishness, malaise and anorexia. On examination he is found to be slightly icteric and has a low-grade fever. The pharynx is slightly infected. The edge of the liver is felt just below the right costal margin. The spleen is easily palpable 3 cm. below the left costal margin. Lymphadenopathy is detected in the posterior cervical and inguinal areas.*

   The most likely diagnosis is:
   A. infectious hepatitis
   B. leptospirosis
   C. infectious mononucleosis
   D. lymphoma
   E. serum hepatitis

10. *The diagnosis can usually be confirmed by:*
    A. blood smear stained for spirochaetes
    B. lymph node biopsy
    C. blood smear for cell count and morphology
    D. lymph node biopsy
    E. liver function tests

11. *An obese 38-year-old female, previously well had been taking an unknown medication for approximately 2 weeks. She presents with sudden onset of fever, chills and slight upper quadrant pain. On examination, the patient is febrile and icteric. The liver edge is palpable and there is right upper quadrant tenderness. The spleen is not palpable. Routine laboratory investigation reveals a normal haemoglobin and a white blood cell count of 11,000 with 8% eosinophils. The most likely diagnosis is:*
    A. drug induced cholestatic jaundice
    B. infectious hepatitis
    C. choledocholithiasis
    D. drug induced hemolytic jaundice
    E. acute cholecystitis

12. *Which of the following liver function test patterns would best fit the clinical picture:*

    A. slightly elevated alkaline phosphatase, SGOT, SGPT and LDH
    B. absent urine urobilinogen, moderately elevated phosphatase, slightly elevated SGOT and SGPT
    C. trace urine urobilinogen, slightly elevated alkaline phosphatase, markedly elevated SGOT and SGPT
    D. moderately elevated alkaline phosphatase, SGOT, SGPT and LDH
    E. 4 + urine urobilinogen, normal alkaline phosphatase, SGOT and SGPT

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Answers

1. D.
   A number of dye excretion tests – among them bromsulphalein, Rose bengal and indocyanine green provide the most sensitive indices of hepatic circulation and of parenchymal and excretory functions. Because of their sensitivity, the dye tests are usually restricted to cases where liver function studies are normal in the face of suspected liver disease.

2. C.
   The two most important factors in the production of ascites are failure of the liver to synthesize albumin (therefore a lowered plasma osmotic pressure), and portal venous hypertension. The interchange of fluid between blood and tissue spaces depends on an equilibrium which may be stated: *plasma colloid osmotic pressure – ascitic colloid osmotic pressure = portal capillary pressure – intraabdominal hydrostatic pressure.*

3. E.
   Anemia of chronic liver disease depends on many factors among which are hypersplenism, chronic blood loss and iron deficiency, hemolysis, nutritional deficiency and bone marrow depression when liver disease is active.

4. D.
   Dullness to percussion due to fullness in the flanks is the earliest sign and can be detected when approximately 2 litres of fluid is present. Abdominal distension is due to fluid and also to air in dilated intestinal loops.

5. E.
   After 40 years of age, the commonest cause of jaundice is carcinoma. Between 40 and 60 years of age, the relative incidence of causes of jaundice is as follows:

carcinoma	40%
cirrhosis	20%
hepatitis	20%
choledocholithiasis	15%
pre-hepatic disorders	5%

6. B.
   The reaction of halothane is indistinguishable from acute virus hepatitis clinically, or by biochemical and histological methods currently available.
   Other drugs implicated in this type of reaction are iproniazid and methoxyflurane (penthiane).

7. D.
   Morphine derivatives and paraldehyde are absolutely contraindicated for sedation. The phenothiazine derivatives have proved useful when sedation in such cases is required.

8. A.
   The emergency management concentrates on maintaining the patient in optimal condition until more definitive action can be undertaken. It consists specifically of the administration of whole blood or other fluids to correct or prevent shock and to maintain haematocrit at a reasonable level. An adequate airway is also mandatory but does not necessarily require intubation.

9. C.
10. C.
    Splenomegaly and lymphadenopathy may be prominent features of infectious mononucleosis and help distinguish the jaundice of this disease from that of infectious or serum hepatitis. The diagnosis can usually be confirmed by a WBC count which shows a relative lymphocytosis and atypical lymphocytes.

11. A.
12. B.
    The history of drug ingestion followed by sudden onset of symptoms and signs suggest a drug induced cholestatic disorder. An eosinophilia helps confirm the diagnosis and a normal haemoglobin helps rule out a hemolytic disorder.

    Since cholestatic jaundice is an obstructive jaundice, alkaline phosphatase will be moderately (or markedly) elevated. However hepatocellular damage in the early stages of disease is usually not marked and SGOT and SGPT will only be slightly elevated.

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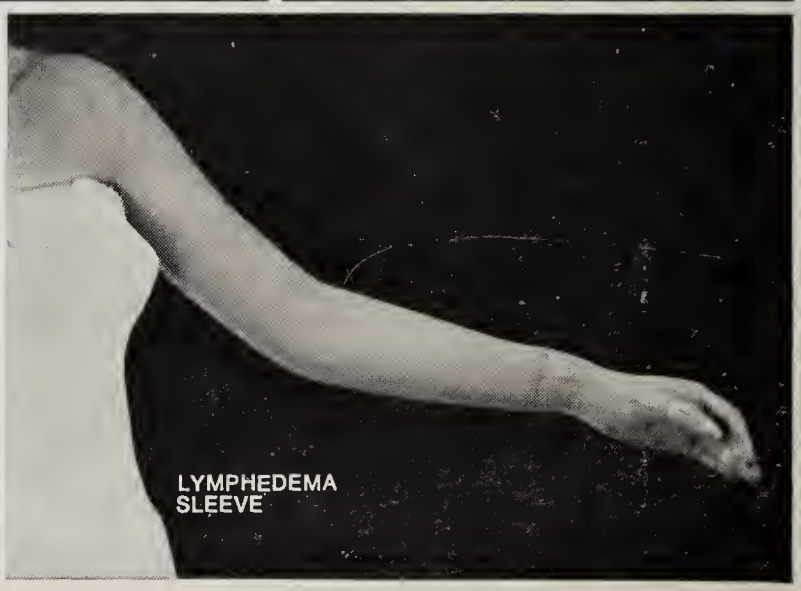

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